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=> s antibod?
L1     2283636 ANTIBOD?

=> s l1 and platelet glycoprotein VI
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L3   ANSWER 1 OF 10  CAPLUS  COPYRIGHT 2002 ACS
2001:12605   Document No. 134:81775  Glycoprotein VI cDNA and protein from
     human and murine blood platelets and their diagnostic and therapeutic
     applications.  Busfield, Samantha J.; Villelal, Jean-luc; Jandrot-Perrus,
     Martine; Vainchencker, William; Gill, Davinder Singh; Qian, Ming Diana;
     Kingsbury, Gillian (Millennium Pharmaceuticals, Inc., USA).  PCT Int.
     Appl. WO 2001000810 A1 20010104, 227 pp.  DESIGNATED STATES: W:  AE, AG,
     AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
     DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
     KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
     NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
     UG, US, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE,
     BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT,
     LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).  CODEN: PIXXD2.
     APPLICATION: WO 2000-US18152 20000630.  PRIORITY: US 1999-345468 19990630;
     US 1999-454824 19991206; US 2000-503387 20000214.
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AB The invention provides isolated cDNA mols. and polypeptide mols. that encode human and murine glycoprotein VI, a platelet membrane glycoprotein that is involved platelet-collagen interactions. The protein initially designated TANGO 268 represents the platelet-expressed collagen receptor glycoprotein VI (GPVI) based on the following evidence: (1) the glycosylated mol. wts. of TANGO 268 and GPVI are identical or similar; (2) both are recognized by anti-GPVI **antibodies** and bind to convulxin; (3) both are preferentially expressed in megakaryocytic cells; (4) both ar predicted to have a single N-glycosylation site; (5) the mol. mass of GPVI upon N- and O-linked glycosylation is .apprx.62 kDa, that of GPVI; (6) two Ig-like domains in TANGO 268 indicates interaction with FcR.gamma.; (7) the absence of a large intracytoplasmic tail suggests that this membrane-bound glycoprotein has no signaling role but assocs. with another member of the Ig family; and (8) TANGO 268 has a charged arginine residue in the transmembrane domain which is also predicted to be present in GPVI. The human gene for GPVI was mapped on radiation hybrid panels to the long arm of chromosome 19, in the region 19q13, syntenic to mouse chromosome 7. The invention also provides antisense nucleic acid mols., expression vectors contg. the nucleic acid mols. of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid mol. of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and **antibodies**. Diagnostic, screening and therapeutic methods utilizing compns. of the invention are also provided.

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L1 2283636 S ANTIBOD?
L2 22 S L1 AND PLATELET GLYCOPROTEIN VI
L3 10 DUP REMOVE L2 (12 DUPLICATES REMOVED)

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L3 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2002 ACS
2001:12605 Document No. 134:81775 Glycoprotein VI cDNA and protein from human and murine blood platelets and their diagnostic and therapeutic applications. Busfield, Samantha J.; Villelal, Jean-luc; Jandrot-Perrus, Martine; Vainchencker, William; Gill, Davinder Singh; Qian, Ming Diana; Kingsbury, Gillian (Millennium Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2001000810 A1 20010104, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US18152 20000630. PRIORITY: US 1999-345468 19990630; US 1999-454824 19991206; US 2000-503387 20000214.

AB The invention provides isolated cDNA mols. and polypeptide mols. that encode human and murine glycoprotein VI, a platelet membrane glycoprotein that is involved platelet-collagen interactions. The protein initially designated TANGO 268 represents the platelet-expressed collagen receptor glycoprotein VI (GPVI) based on the following evidence: (1) the glycosylated mol. wts. of TANGO 268 and GPVI are identical or similar; (2) both are recognized by anti-GPVI **antibodies** and bind to convulxin; (3) both are preferentially expressed in megakaryocytic cells; (4) both ar predicted to have a single N-glycosylation site; (5) the mol. mass of GPVI upon N- and O-linked glycosylation is .apprx.62 kDa, that of

GPVI; (6) two Ig-like domains in TANGO 268 indicates interaction with FcR.gamma.; (7) the absence of a large intracytoplasmic tail suggests that this membrane-bound glycoprotein has no signaling role but assocs. with another member of the Ig family; and (8) TANGO 268 has a charged arginine residue in the transmembrane domain which is also predicted to be present in GPVI. The human gene for GPVI was mapped on radiation hybrid panels to the long arm of chromosome 19, in the region 19q13, syntenic to mouse chromosome 7. The invention also provides antisense nucleic acid mols., expression vectors contg. the nucleic acid mols. of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid mol. of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and **antibodies**. Diagnostic, screening and therapeutic methods utilizing compns. of the invention are also provided.

L3 ANSWER 2 OF 10 MEDLINE DUPLICATE 1

2001258216 Document Number: 21102921. PubMed ID: 11181698. Long-term antithrombotic protection by in vivo depletion of **platelet glycoprotein VI** in mice. Nieswandt B; Schulte V; Bergmeier W; Mokhtari-Nejad R; Rackebrandt K; Cazenave J P; Ohlmann P; Gachet C; Zirngibl H. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, 42117 Wuppertal, Germany.. nieswand@klinikum-wuppertal.de) . JOURNAL OF EXPERIMENTAL MEDICINE, (2001 Feb 19) 193 (4) 459-69. Journal code: I2V; 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque and activation of platelets on the subendothelial layers in the disrupted plaque. The extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer; therefore, a selective inhibition of the collagen activation pathway in platelets may provide strong antithrombotic protection while preserving other platelet functions. Here we demonstrate that treatment of mice with a monoclonal **antibody** against the activating platelet collagen receptor glycoprotein VI (GPVI; JAQ1) results in specific depletion of the receptor from circulating platelets and abolished responses of these cells to collagen and collagen-related peptides (CRPs). JAQ1-treated mice were completely protected for at least 2 wk against lethal thromboembolism induced by infusion of a mixture of collagen (0.8 mg/kg) and epinephrine (60 microg/ml). The tail bleeding times in JAQ1-treated mice were only moderately increased compared with control mice probably because the treatment did not affect platelet activation by other agonists such as adenosine diphosphate or phorbol myristate acetate. These results suggest that GPVI might become a target for long-term prophylaxis of ischemic cardiovascular diseases and provide the first evidence that it is possible to specifically deplete an activating glycoprotein receptor from circulating platelets in vivo.

L3 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2002 ACS

2000:569621 Document No. 133:250251 Expression and function of the mouse collagen receptor glycoprotein VI is strictly dependent on its association with the FcR.gamma. chain. Nieswandt, Bernhard; Bergmeier, Wolfgang; Schulte, Valerie; Rackebrandt, Kirsten; Gessner, J. Engelbert; Zirngibl, Hubert (Department of Molecular Oncology, General Surgery, University of Witten-Herdecke, Wuppertal, 42283, Germany). J. Biol. Chem., 275(31), 23998-24002 (English) 2000. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Platelet glycoprotein (GP) VI has been proposed as the major collagen receptor for activation of human platelets. Human GPVI belongs to the Ig superfamily and is noncovalently assocd. with the FcR.gamma. chain that is involved in signaling through the receptor. In mice, similar mechanisms seem to exist, as platelets from FcR.gamma. chain-deficient mice do not aggregate in response to collagen. However, the activating collagen receptor on mouse platelets has not been definitively identified. In the current study the authors examd. the function and in vivo expression of

GPVI in control and FcR.gamma. chain-deficient mice with the first monoclonal **antibody** against GPVI (JAQ1). On wild type platelets, JAQ1 inhibited platelet aggregation induced by collagen but not PMA or thrombin. Crosslinking of bound JAQ1, on the other hand, induced aggregation of wild type but not FcR.gamma. chain-deficient platelets. JAQ1 stained platelets and megakaryocytes from wild type but not FcR.gamma. chain-deficient mice. Furthermore, JAQ1 recognized GPVI (approx. 60 kDa) in immunopptn. and Western blot expts. with wild type but not FcR.gamma. chain-deficient platelets. These results strongly suggest that GPVI is the collagen receptor responsible for platelet activation in mice and demonstrate that the assocn. with the FcR.gamma. chain is crit. for its expression and function.

L3 ANSWER 4 OF 10 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

2000081456 EMBASE Distinct contributions of glycoprotein VI and .alpha.2.beta.1 integrin to the induction of platelet protein tyrosine phosphorylation and aggregation. Kamiguti A.S.; Theakston R.D.G.; Watson S.P.; Bon C.; Laing G.D.; Zuzel M.. A.S. Kamiguti, Department of Haematology, Royal Liverpool Hospital, University of Liverpool, Liverpool, United Kingdom. aurakami@liverpool.ac.uk. Archives of Biochemistry and Biophysics 374/2 (356-362) 15 Feb 2000.
Refs: 39.
ISSN: 0003-9861. CODEN: ABBIA4. Pub. Country: United States. Language: English. Summary Language: English.

AB Platelet activation by collagen depends principally on two receptors, .alpha.2.beta.1 integrin (GPIa-IIa) and GPVI. During this activation, the nonreceptor protein tyrosine kinase pp72(syk) is rapidly phosphorylated, but the precise contribution of .alpha.2.beta.1 integrin and GPVI to signaling for this phosphorylation is not clear. We have recently found that proteolysis of platelet .alpha.2.beta.1 integrin by the snake venom metalloproteinase, jararhagin, results in inhibition of collagen-induced platelet aggregation and pp72(syk) phosphorylation. In order to verify whether the treatment of platelets with jararhagin had any effect on GPVI signaling, in this study we stimulated platelets treated with either jararhagin or anti-.alpha.2.beta.1 **antibody** with two GPVI agonists, an **antibody** to GPVI and convulxin. Platelet shape change and phosphorylation of pp72(syk) by both GPVI agonists was preserved, as was the structure and function of GPVI shown by 125I-labeled convulxin binding to immunoprecipitated GPVI from jararhagin-treated platelets. In contrast, defective platelet aggregation in response to GPVI agonists occurred in both jararhagin-treated and .alpha.2.beta.1-blocked platelets. This apparent cosignaling role of .alpha.2.beta.1 integrin for platelet aggregation suggests the possibility of a topographical association of this integrin with GPVI. We found that both platelet .alpha.2.beta.1 integrin and GPVI coimmunoprecipitated with .alpha.(11b).beta.3 integrin. Since platelet aggregation requires activation of .alpha.(11b).beta.3, integrin, defective aggregation in the absence of .alpha.2.beta.1 suggests that this receptor may provide a signaling link between GPVI and .alpha.(11b).beta.3. Our study therefore demonstrates that platelet signaling leading to pp72(syk) phosphorylation initiated with GPVI engagement by either convulxin or GPVI **antibody** does not depend on .alpha.2.beta.1 integrin. However, .alpha.(11b).beta.3 integrin may, in this model, require functional .alpha.2.beta.1 integrin for its activation. (C) 2000 Academic Press.

L3 ANSWER 5 OF 10 MEDLINE DUPLICATE 2

2000474813 Document Number: 20284011. PubMed ID: 10822077. Cloning and expression of the platelet-specific collagen receptor glycoprotein VI. Miura Y; Ohnuma M; Jung S M; Moroi M. (Department of Protein Biochemistry, Institute of Life Science, Kurume University, Fukuoka, Japan.) THROMBOSIS RESEARCH, (2000 May 15) 98 (4) 301-9. Journal code: VRN; 0326377. ISSN: 0049-3848. Pub. country: United States. Language: English.

AB **Platelet glycoprotein VI** (GP VI) was purified from platelet membranes and its internal amino acid sequences were determined. The cloned cDNA of GP VI indicates an open reading frame

coding for 20 amino acid signal sequences and a mature protein of 319 amino acids. Its extracellular region has two Ig-like domains and a mucin-like, Ser/Thr-rich region, suggesting that GP VI is a member of the paired Ig-like receptor family. GP VI-transfected cells contained convulxin-(reactive) and **antibody** against recombinant GP VI-reactive protein bands that migrated at the same position as platelet GP VI in SDS/PAGE-electroblotting. These data indicate that the protein deduced from the cloned cDNA corresponds to platelet GP VI.

L3 ANSWER 6 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:311599 Document No.: PREV200100311599. Long-term antithrombotic protection by irreversible inactivation of **platelet glycoprotein VI** in mice. Nieswandt, Bernhard (1); Schulte, Valerie (1); Bergmeier, Wolfgang (1); Mokhtari-Nejad, Rabee (1); Cazenave, Jean P.; Gachet, Christian; Zirngibl, Hubert (1). (1) Molecular Oncology, Witten/Herdecke University, Wuppertal Germany. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 269a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology. ISSN: 0006-4971. Language: English. Summary Language: English.

AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque followed by deposition and activation of platelets on the subendothelial layers in the disrupted plaque. Because the extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer, a selective inhibition of the collagen activation pathway in platelets may provide strong antithrombotic protection while preserving other platelet functions. Growing evidence suggests that platelet glycoprotein (GP) VI is the major collagen receptor for platelet activation making this receptor a good candidate for such a specific inhibition. In the current study, we have investigated the antithrombotic effects of the first monoclonal **antibody** (mAb) against mouse GPVI (JAQ1, Nieswandt et al; 2000, J Biol Chem, 275(31):23998-24002). Injection of 100 mug JAQ1 only had mild and transient effects on platelet counts with a maximum drop of approximately 34 +- 7.4 % on day 1 and a return to normal after 2-3 days. JAQ1 pretreated mice were completely protected against lethal thromboembolism induced by infusion of a mixture of collagen (0.8 mg/kg) and epinephrine (60 mug/kg) for at least two weeks (100% survivors on days 3, 7, and 14 after mAb injection, n=8 per group, 5% survivors in the control group, n=20). Aggregometric and flow cytometric studies demonstrated that platelets from JAQ1 treated mice were completely resistant against activation with high concentrations of collagen (up to 50 mug/ml) and collagen related peptides (up to 100 mug/ml) ex vivo on days 3, 7, and 14. In JAQ1 treated mice, GPVI was not detectable in a Western blot analysis of platelet lysates for minimally two weeks, suggesting irreversible inactivation (or degradation) of the receptor on circulating platelets. In contrast to collagen, other agonists, such as ADP or platelet aggregating agents, such as PMA induced normal activation and aggregation of these platelets. Consequently, the tail bleeding times were only moderately increased in anti-GPVI treated mice compared to control mice on day 3, 7, and 14. These results establish GPVI as an attractive target for long-term antithrombotic therapy.

L3 ANSWER 7 OF 10 MEDLINE DUPLICATE 3
1999273390 Document Number: 99273390. PubMed ID: 10341844.
Collagen-platelet interaction: Gly-Pro-Hyp is uniquely specific for platelet Gp VI and mediates platelet activation by collagen. Knight C G; Morton L F; Onley D J; Peachey A R; Ichinohe T; Okuma M; Farndale R W; Barnes M J. (Biochemistry Department, Cambridge University, UK.) CARDIOVASCULAR RESEARCH, (1999 Feb) 41 (2) 450-7. Journal code: COR; 0077427. ISSN: 0008-6363. Pub. country: Netherlands. Language: English.

AB OBJECTIVE: Peptides consisting of a repeat Gly Pro-Hyp sequence are potent platelet agonists. The aim of this study was: (1) to examine the specificity of this sequence for platelet activation; (2) to confirm its recognition by **platelet glycoprotein VI**; and

(3) to assess with suitable peptides the relative importance of glycoprotein VI and integrin alpha 2 beta 1 in platelet activation by collagen. METHODS: Peptides were synthesized by standard Fmoc chemistry and tested for their ability to support adhesion of human platelets and HT 1080 cells, induce platelet aggregation, bind integrin alpha 2 subunit A-domain and to cause tyrosine phosphorylation of platelet proteins. RESULTS: (1) Peptides consisting of a repeat Gly-Pro-Pro, Gly-Pro-Ala or Gly-Pro-Arg sequence exhibited little if any platelet-reactivity. (2) The platelet-reactive peptide consisting of a repeating Gly-Pro-Hyp sequence failed to induce tyrosine phosphorylation in glycoprotein VI-deficient platelets. Platelet adhesion to this peptide was inhibited by intact anti-glycoprotein VI **antibody** and its Fab fragment. The latter inhibited aggregation by the peptide and fibres of both collagens I and III. (3) A peptide containing a 15-mer alpha 2 beta 1-binding sequence in a repeat Gly-Pro-Pro structure supported alpha 2 beta 1-mediated platelet and HT 1080 cell adhesion and bound alpha 2 A-domain, but failed to activate platelets or to induce tyrosine phosphorylation. Conversely, a peptide containing this sequence but with an essential Glu replaced by Ala and inserted in a repeat Gly-Pro-Hyp structure did not recognize alpha 2 beta 1, but was highly platelet activatory. CONCLUSIONS: Platelet activation by collagen involves the highly-specific recognition of the Gly-Pro-Hyp sequence by **platelet glycoprotein VI**. Recognition of alpha 2 beta 1 is insufficient to cause activation. Interaction between collagen and glycoprotein VI is unique since Gly-Pro-Hyp is common in collagens but occurs rarely in other proteins, and glycoprotein VI may be expressed solely by platelets. This sequence could provide a basis for a highly-specific anti-thrombotic reagent to control thrombosis associated with plaque rupture.

L3 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2002 ACS

1998:453055 Document No. 129:173552 Convulxin-induced platelet adhesion and aggregation: involvement of glycoproteins VI and IaIIa. Jandrot-Perrus, M.; Lagrue, A. H.; Leduc, M.; Okuma, M.; Bon, C. (Laboratoire de Recherche sur l'Hemostase et la Thrombose, Faculte de Medecine Xavier Bichat, Paris, 75870, Fr.). Platelets, 9(3/4), 207-211 (English) 1998. CODEN: PLTEEF. ISSN: 0953-7104. Publisher: Carfax Publishing Ltd..

AB The interaction of convulxin (Cvx), a 72-kDa glycoprotein isolated from the venom of Crotalus durissus terrificus with human blood platelets was studied. Cvx at low concns. (<100 pM) induced platelet aggregation, dense body secretion, and intracellular Ca2+ mobilization which indicated that Cvx is a potent activator of human platelets. Cvx-induced platelet aggregation and secretion was inhibited by 6F1 an anti-integrin .alpha.2.beta.1 monoclonal **antibody** that was without effect on Ca2+ mobilization. Anti-glycoprotein VI (GPVI) Fab fragments inhibited aggregation, secretion, and Ca2+ mobilization triggered by Cvx. In addn., immobilized Cvx was found to induce divalent cation-independent platelet adhesion in a static system. Platelet adhesion to Cvx was inhibited by anti-GPVI Fab fragments but not by anti-integrin .alpha.2.beta.1. Cvx was shown to bind to a 57-kDa protein that was identified as GPVI. Altogether, these results indicated that GPVI behaves as a receptor for Cvx, whereas integrin .alpha.2.beta.1 could play a regulatory role in Cvx-induced platelet aggregation. Cvx and collagen interaction with platelets thus appears to share some characteristics but to also have specific properties.

L3 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2002 ACS

1995:962205 Document No. 124:26392 Cyclic AMP-insensitive activation of c-Src and Syk protein-tyrosine kinases through platelet membrane glycoprotein VI. Ichinohe, Tatsuo; Takayama, Hiroshi; Ezumi, Yasuharu; Yanagi, Shigeru; Yamamura, Hirohei; Okuma, Minoru (Fac. Med., Kyoto Univ., Kyoto, 606, Japan). J. Biol. Chem., 270(47), 28029-36 (English) 1995. CODEN: JBCHA3. ISSN: 0021-9258.

AB Platelet glycoprotein (GP) VI is a so-far uncharacterized 62-kDa membrane protein, whose deficiency results in selective impairment in collagen-induced platelet aggregation. The group previously reported a

human polyclonal **antibody** (anti-p62 IgG) that induces activation of normal, but not of GPVI-deficient, platelets in an Fc-independent manner. The F(ab')2 fragments of this **antibody** (F(ab')2-anti-p62) stimulated tyrosine phosphorylation of numerous proteins, which was not prevented even in the presence of cAMP-increasing agents such as prostacyclin. Pretreatment of platelets with the protein-tyrosine kinase (PTK) inhibitor tyrphostin A47 completely abolished F(ab')2-anti-p62-induced platelet aggregation in parallel with dose-dependent inhibition of protein-tyrosine phosphorylation, indicating an essential requirement of PTK activity for generating GPVI-mediated signaling. The authors found that two cytosolic PTKs, c-Src and Syk, became rapidly activated in response to F(ab')2-anti-p62 in a way insensitive to elevation of cAMP. In contrast, in the presence of prostacyclin, F(ab')2-anti-p62 did not stimulate tyrosine phosphorylation of the focal adhesion kinase. CAMP-insensitive activation of c-Src and Syk was also obsd. in collagen-but not thrombin-stimulated platelets. Moreover, either F(ab')2-anti-p62 or collagen stimulated cAMP-insensitive tyrosine phosphorylation of phospholipase C-.gamma.2. These results indicate that the receptor-mediated activation of several PTKs in platelets is regulated through a cAMP-sensitive or -insensitive mechanism depending on the nature of each stimulus, and also suggest that GPVI engagement is coupled to cAMP-insensitive activation of c-Src and Syk accompanied by tyrosine phosphorylation of numerous substrates including phospholipase C-.gamma.2 in a manner similar to collagen stimulation.

L3 ANSWER 10 OF 10 SCISEARCH COPYRIGHT 2002 ISI (R)
95:80020 The Genuine Article (R) Number: QB057. PLATELETS WITH 10-PERCENT OF THE NORMAL AMOUNT OF GLYCOPROTEIN-VI HAVE AN IMPAIRED RESPONSE TO COLLAGEN THAT RESULTS IN A MILD BLEEDING TENDENCY. ARAI M (Reprint); YAMAMOTO N; MOROI M; AKAMATSU N; FUKUTAKE K; TANOUE K. TOKYO MED COLL, DEPT CLIN PATHOL, SHINJUKU KU, 6-7-1 NISHISHINJUKU, TOKYO 160, JAPAN (Reprint); TOKYO METROPOLITAN INST MED SCI, DEPT CARDIOVASC RES, TOKYO 113, JAPAN; KURUME UNIV, INST LIFE SCI, DEPT PROT BIOCHEM, KURUME, FUKUOKA, JAPAN. BRITISH JOURNAL OF HAEMATOLOGY (JAN 1995) Vol. 89, No. 1, pp. 124-130. ISSN: 0007-1048. Pub. country: JAPAN. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Platelet glycoprotein VI** (GPVI), a 62 kD membrane protein, has been identified as one of the platelet receptors for collagen, since GPVI-deficient platelets exhibit abnormal responses to collagen and an abnormal bleeding tendency. We report a female patient with a mild bleeding history whose platelets expressed 10% GPVI of normal platelets. Shape change, aggregation and ATP release of the patient's platelets were completely absent in response to 1-5 mu g/ml collagen but present normally in response to ADP and Ca2+ ionophore A23187. Adhesion of the patient's platelets to coated collagen was mildly affected (40-60% of normal platelets) in spite of only 10% expression of GPVI. Flow cytometrical studies revealed that the patient's platelets expressed normal amounts of the GPIa/IIa complex. These results suggest that platelet GPVI is less involved in adhesion to collagen than shape change and aggregation induced by collagen.

=> s platelet collagen receptor antibod?
L4 0 PLATELET COLLAGEN RECEPTOR ANTIBOD?

=> d his

(FILE 'HOME' ENTERED AT 15:45:17 ON 14 MAR 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:45:50 ON 14 MAR 2002
L1 2283636 S ANTIBOD?
L2 22 S L1 AND PLATELET GLYCOPROTEIN VI
L3 10 DUP REMOVE L2 (12 DUPLICATES REMOVED)
L4 0 S PLATELET COLLAGEN RECEPTOR ANTIBOD?

```
=> s l3 and human
L5            6 L3 AND HUMAN

=> dup remove l5
PROCESSING COMPLETED FOR L5
L6            6 DUP REMOVE L5 (0 DUPLICATES REMOVED)

=> s l6 and monoclonal
L7            3 L6 AND MONOCLONAL

=> dup remove l7
PROCESSING COMPLETED FOR L7
L8            3 DUP REMOVE L7 (0 DUPLICATES REMOVED)

=> d l8 1-3 cbib abs

L8   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2002 ACS
2001:12605  Document No. 134:81775  Glycoprotein VI cDNA and protein from
     human and murine blood platelets and their diagnostic and
     therapeutic applications.  Busfield, Samantha J.; Villelal, Jean-luc;
     Jandrot-Perrus, Martine; Vainchencker, William; Gill, Davinder Singh;
     Qian, Ming Diana; Kingsbury, Gillian (Millennium Pharmaceuticals, Inc.,
     USA).  PCT Int. Appl. WO 2001000810 A1 20010104, 227 pp.  DESIGNATED
     STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN,
     CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
     MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ,
     TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU,
     TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR,
     GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.
     (English).  CODEN: PIXXD2.  APPLICATION: WO 2000-US18152 20000630.
     PRIORITY: US 1999-345468 19990630; US 1999-454824 19991206; US 2000-503387
     20000214.
AB   The invention provides isolated cDNA mols. and polypeptide mols. that
     encode human and murine glycoprotein VI, a platelet membrane
     glycoprotein that is involved platelet-collagen interactions.  The protein
     initially designated TANGO 268 represents the platelet-expressed collagen
     receptor glycoprotein VI (GPVI) based on the following evidence: (1) the
     glycosylated mol. wts. of TANGO 268 and GPVI are identical or similar; (2)
     both are recognized by anti-GPVI antibodies and bind to
     convulxin; (3) both are preferentially expressed in megakaryocytic cells;
     (4) both ar predicted to have a single N-glycosylation site; (5) the mol.
     mass of GPVI upon N- and O-linked glycosylation is .apprx.62 kDa, that of
     GPVI; (6) two Ig-like domains in TANGO 268 indicates interaction with
     FcR.gamma.; (7) the absence of a large intracytoplasmic tail suggests that
     this membrane-bound glycoprotein has no signaling role but assocs. with
     another member of the Ig family; and (8) TANGO 268 has a charged arginine
     residue in the transmembrane domain which is also predicted to be present
     in GPVI.  The human gene for GPVI was mapped on radiation hybrid
     panels to the long arm of chromosome 19, in the region 19q13, syntenic to
     mouse chromosome 7.  The invention also provides antisense nucleic acid
     mols., expression vectors contg. the nucleic acid mols. of the invention,
     host cells into which the expression vectors have been introduced, and
     non-human transgenic animals in which a nucleic acid mol. of the
     invention has been introduced or disrupted.  The invention still further
     provides isolated polypeptides, fusion polypeptides, antigenic peptides
     and antibodies.  Diagnostic, screening and therapeutic methods
     utilizing compns. of the invention are also provided.

L8   ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2002 ACS
2000:569621  Document No. 133:250251  Expression and function of the mouse
     collagen receptor glycoprotein VI is strictly dependent on its association
     with the FcR.gamma. chain.  Nieswandt, Bernhard; Bergmeier, Wolfgang;
     Schulte, Valerie; Rackebrandt, Kirsten; Gessner, J. Engelbert; Zirngibl,
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Hubert (Department of Molecular Oncology, General Surgery, University of Witten-Herdecke, Wuppertal, 42283, Germany). J. Biol. Chem., 275(31), 23998-24002 (English) 2000. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Platelet glycoprotein (GP) VI has been proposed as the major collagen receptor for activation of **human** platelets. **Human** GPVI belongs to the Ig superfamily and is noncovalently assocd. with the FcR.gamma. chain that is involved in signaling through the receptor. In mice, similar mechanisms seem to exist, as platelets from FcR.gamma. chain-deficient mice do not aggregate in response to collagen. However, the activating collagen receptor on mouse platelets has not been definitively identified. In the current study the authors examd. the function and in vivo expression of GPVI in control and FcR.gamma. chain-deficient mice with the first **monoclonal antibody** against GPVI (JAQ1). On wild type platelets, JAQ1 inhibited platelet aggregation induced by collagen but not PMA or thrombin. Crosslinking of bound JAQ1, on the other hand, induced aggregation of wild type but not FcR.gamma. chain-deficient platelets. JAQ1 stained platelets and megakaryocytes from wild type but not FcR.gamma. chain-deficient mice. Furthermore, JAQ1 recognized GPVI (approx. 60 kDa) in immunopptn. and Western blot expts. with wild type but not FcR.gamma. chain-deficient platelets. These results strongly suggest that GPVI is the collagen receptor responsible for platelet activation in mice and demonstrate that the assocn. with the FcR.gamma. chain is crit. for its expression and function.

L8 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2002 ACS

1998:453055 Document No. 129:173552 Convulxin-induced platelet adhesion and aggregation: involvement of glycoproteins VI and IaIIa. Jandrot-Perrus, M.; Lagrue, A. H.; Leduc, M.; Okuma, M., Bon, C. (Laboratoire de Recherche sur l'Hemostase et la Thrombose, Faculte de Medecine Xavier Bichat, Paris, 75870, Fr.). Platelets, 9(3/4), 207-211 (English) 1998. CODEN: PLTEEF. ISSN: 0953-7104. Publisher: Carfax Publishing Ltd..

AB The interaction of convulxin (Cvx), a 72-kDa glycoprotein isolated from the venom of Crotalus durissus terrificus with **human** blood platelets was studied. Cvx at low concns. (<100 pM) induced platelet aggregation, dense body secretion, and intracellular Ca2+ mobilization which indicated that Cvx is a potent activator of **human** platelets. Cvx-induced platelet aggregation and secretion was inhibited by 6F1 an anti-integrin .alpha.2.beta.1 **monoclonal antibody** that was without effect on Ca2+ mobilization. Anti-glycoprotein VI (GPVI) Fab fragments inhibited aggregation, secretion, and Ca2+ mobilization triggered by Cvx. In addn., immobilized Cvx was found to induce divalent cation-independent platelet adhesion in a static system. Platelet adhesion to Cvx was inhibited by anti-GPVI Fab fragments but not by anti-integrin .alpha.2.beta.1. Cvx was shown to bind to a 57-kDa protein that was identified as GPVI. Altogether, these results indicated that GPVI behaves as a receptor for Cvx, whereas integrin .alpha.2.beta 1 could play a regulatory role in Cvx-induced platelet aggregation. Cvx and collagen interaction with platelets thus appears to share some characteristics but to also have specific properties.

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=> s l3 and chimeric
L9            0 L3 AND CHIMERIC

=> s l3 and humanized
L10           1 L3 AND HUMANIZED

=> d l10 cbib abs
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L10 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS

2001:12605 Document No. 134:81775 Glycoprotein VI cDNA and protein from human and murine blood platelets and their diagnostic and therapeutic

applications. Busfield, Samantha J.; Villelal, Jean-luc; Jandrot-Perrus, Martine; Vainchencker, William; Gill, Davinder Singh; Qian, Ming Diana; Kingsbury, Gillian (Millennium Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2001000810 A1 20010104, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US18152 20000630. PRIORITY: US 1999-345468 19990630; US 1999-454824 19991206; US 2000-503387 20000214.

AB The invention provides isolated cDNA mols. and polypeptide mols. that encode human and murine glycoprotein VI, a platelet membrane glycoprotein that is involved platelet-collagen interactions. The protein initially designated TANGO 268 represents the platelet-expressed collagen receptor glycoprotein VI (GPVI) based on the following evidence: (1) the glycosylated mol. wts. of TANGO 268 and GPVI are identical or similar; (2) both are recognized by anti-GPVI **antibodies** and bind to convulxin; (3) both are preferentially expressed in megakaryocytic cells; (4) both ar predicted to have a single N-glycosylation site; (5) the mol. mass of GPVI upon N- and O-linked glycosylation is .apprx.62 kDa, that of GPVI; (6) two Ig-like domains in TANGO 268 indicates interaction with FcR.gamma.; (7) the absence of a large intracytoplasmic tail suggests that this membrane-bound glycoprotein has no signaling role but assocs. with another member of the Ig family; and (8) TANGO 268 has a charged arginine residue in the transmembrane domain which is also predicted to be present in GPVI. The human gene for GPVI was mapped on radiation hybrid panels to the long arm of chromosome 19, in the region 19q13, syntenic to mouse chromosome 7. The invention also provides antisense nucleic acid mols., expression vectors contg. the nucleic acid mols. of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid mol. of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and **antibodies**. Diagnostic, screening and therapeutic methods utilizing compns. of the invention are also provided.

```
=> s Fc fusion GPVI
L11           0 FC FUSION GPVI

=> s Fc fusion polypeptide
L12           0 FC FUSION POLYPEPTIDE

=> s Fc chimeric
L13         156 FC CHIMERIC

=> s l13 and GPVI
L14           0 L13 AND GPVI

=> s busfield s?/au or villeval j?/au or jandrot-perrus m?/au or vainchencker w?/au
L15         534 BUSFIELD S?/AU OR VILLEVAL J?/AU OR JANDROT-PERRUS M?/AU OR
                VAINCHENCKER W?/AU

=> s l15 and platelet glycoprotein VI or "GPVI"
L16         436 L15 AND PLATELET GLYCOPROTEIN VI OR "GPVI"

=> s l16 and antibody
L17         137 L16 AND ANTIBODY

=> s l17 and monoclonal or human or humanized or chimeric or polyclonal
SYSTEM LIMITS EXCEEDED - SEARCH ENDED
   1 FILES SEARCHED...
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   3 FILES SEARCHED...
COMMAND INTERRUPTED
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help
Desk by telephone or via SEND in the STNMAIL file.

=> dup remove l17
PROCESSING COMPLETED FOR L17
COMMAND INTERRUPTED
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help
Desk by telephone or via SEND in the STNMAIL file.

=> d his

     (FILE 'HOME' ENTERED AT 15:45:17 ON 14 MAR 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:45:50 ON
     14 MAR 2002
L1      2283636 S ANTIBOD?
L2           22 S L1 AND PLATELET GLYCOPROTEIN VI
L3           10 DUP REMOVE L2 (12 DUPLICATES REMOVED)
L4            0 S PLATELET COLLAGEN RECEPTOR ANTIBOD?
L5            6 S L3 AND HUMAN
L6            6 DUP REMOVE L5 (0 DUPLICATES REMOVED)
L7            3 S L6 AND MONOCLONAL
L8            3 DUP REMOVE L7 (0 DUPLICATES REMOVED)
L9            0 S L3 AND CHIMERIC
L10           1 S L3 AND HUMANIZED
L11           0 S FC FUSION GPVI
L12           0 S FC FUSION POLYPEPTIDE
L13         156 S FC CHIMERIC
L14           0 S L13 AND GPVI
L15         534 S BUSFIELD S?/AU OR VILLEVAL J?/AU OR JANDROT-PERRUS M?/AU OR V
L16         436 S L15 AND PLATELET GLYCOPROTEIN VI OR "GPVI"
L17         137 S L16 AND ANTIBODY

=> dup remove l17
PROCESSING COMPLETED FOR L17
COMMAND INTERRUPTED
If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help
Desk by telephone or via SEND in the STNMAIL file.

=> s l16 and antibod?
L20         137 L16 AND ANTIBOD?

=> dup remove l20
PROCESSING COMPLETED FOR L20
L21          44 DUP REMOVE L20 (93 DUPLICATES REMOVED)

=> d l21 1-44 cbib abs

L21  ANSWER 1 OF 44     MEDLINE                              DUPLICATE 1
2002075876  Document Number: 21659770.    PubMed ID: 11723134.   The platelet
     receptor GPVI mediates both adhesion and signaling responses to
     collagen in a receptor density-dependent fashion. Chen Hong; Locke Darren;
     Liu Ying; Liu Changdong; Kahn Mark L. (Department of Medicine, University
     of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. ) JOURNAL OF
     BIOLOGICAL CHEMISTRY, (2002 Jan 25) 277 (4) 3011-9. Journal code:
     2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB   The platelet response to collagen is a primary event in hemostasis and
     thrombosis, but the precise roles of the numerous identified platelet
     collagen receptors remain incompletely defined. Attention has recently
     focused on glycoprotein VI (GPVI), a receptor that is expressed
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on platelets in association with a signaling adapter, the Fc receptor gamma chain (Fc Rgamma). Genetic and pharmacologic loss of **GPVI** function results in loss of collagen signaling in platelets, but studies to date have failed to demonstrate that **GPVI**-Fc Rgamma expression is sufficient to confer collagen signaling responses. These results have led to the hypothesis that collagen responses mediated by **GPVI**-Fc Rgamma may require the collagen-binding integrin alpha2beta1 as a co-receptor, but this model has not been supported by a recent study of mouse platelets lacking alpha2beta1. In the present study we have used a novel anti-**GPVI** monoclonal **antibody** to measure the level of **GPVI** on human platelets and to guide the development of **GPVI**-expressing cell lines to assess the role of **GPVI** in mediating platelet collagen responses. **GPVI** receptor density on human platelets appears tightly regulated, is independent from the level of alpha2beta1 expression, and significantly exceeds that on previously characterized **GPVI**-expressing RBL-2H3 cells. Using newly generated **GPVI**-expressing RBL-2H3 cells with receptor densities equivalent to that on human platelets, we demonstrate that **GPVI** expression confers both adhesive and signaling responses to collagen in a graded fashion that is proportional to the **GPVI** receptor density. These results resolve some of the conflicting data regarding **GPVI**-collagen interactions and demonstrate that 1) **GPVI**-Fc Rgamma expression is sufficient to confer both adhesion and signaling responses to collagen, and 2) **GPVI**-mediated collagen responses are receptor density-dependent at the receptor levels expressed on human platelets.

L21 ANSWER 2 OF 44 MEDLINE

2002045004 Document Number: 21628775. PubMed ID: 11756163. Platelet endothelial cell adhesion molecule-1 signaling inhibits the activation of human platelets. Cicmil Milenko; Thomas Joanne M; Leduc Mireille; Bon Cassian; Gibbins Jonathan M. (School of Animal and Microbial Sciences, University of Reading, United Kingdom.) BLOOD, (2002 Jan 1) 99 (1) 137-44. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) is a 130-kd transmembrane glycoprotein and a member of the growing family of receptors with immunoreceptor tyrosine-based inhibitory motifs (ITIMs). PECAM-1 is expressed on platelets, certain T cells, monocytes, neutrophils, and vascular endothelial cells and is involved in a range of cellular processes, though the role of PECAM-1 in platelets is unclear. Cross-linking of PECAM-1 results in phosphorylation of the ITIM allowing the recruitment of signaling proteins that bind by way of Src-homology domain 2 interactions. Proteins that have been implicated in the negative regulation of cellular activation by ITIM-bearing receptors include the tyrosine phosphatases SHP-1 and SHP-2. Tyrosine phosphorylation of immunoreceptor tyrosine-based activatory motif (ITAM)-bearing receptors such as the collagen receptor **GPVI**-Fc receptor gamma-chain complex on platelets leads to activation. Increasing evidence suggests that ITIM- and ITAM-containing receptors may act antagonistically when expressed on the same cell. In this study it is demonstrated that cross-linking PECAM-1 inhibits the aggregation and secretion of platelets in response to collagen and the **GPVI**-selective agonist convulxin. In these experiments thrombin-mediated platelet aggregation and secretion were also reduced, albeit to a lesser degree than for collagen, suggesting that PECAM-1 function may not be restricted to the inhibition of ITAM-containing receptor pathways. PECAM-1 activation also inhibited platelet protein tyrosine phosphorylation stimulated by convulxin and thrombin; this was accompanied by inhibition of the mobilization of calcium from intracellular stores. These data suggest that PECAM-1 may play a role in the regulation of platelet function in vivo.

L21 ANSWER 3 OF 44 CAPLUS COPYRIGHT 2002 ACS

2001:168145 Document No. 134:217195 Platelet membrane glycoprotein VI (**GPVI**) cDNA and protein sequences, and therapeutic uses thereof.

Tandon, Narendra; Sun, Bing; Nakamura, Takashi; Yamamoto, Naomasa (Otsuka Pharmaceutical Co., Ltd., Japan). PCT Int. Appl. WO 2001016321 A1 20010308, 74 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US23975 20000901. PRIORITY: US 1999-PV152197 19990901; US 1999-PV158251 19991008.

AB The present invention comprises a method of purifying platelet membrane glycoprotein VI (**GPVI**), **GPVI** peptides, cDNA and protein sequence, and methods for using **GPVI** and **antibodies** directed against **GPVI**. It was shown that the extracellular domain of **GPVI** has potent anti-thrombotic activity. The invention comprises methods of inhibiting thrombosis by inhibiting platelet aggregation or platelet activation using **antibodies** directed against **GPVI**, or **GPVI** protein, in particular, the extracellular domain of **GPVI**.

L21 ANSWER 4 OF 44 CAPLUS COPYRIGHT 2002 ACS

2001:12605 Document No. 134:81775 Glycoprotein VI cDNA and protein from human and murine blood platelets and their diagnostic and therapeutic applications. **Busfield, Samantha J.**; Villelal, Jean-luc; **Jandrot-Perrus, Martine**; **Vainchencker, William**; Gill, Davinder Singh; Qian, Ming Diana; Kingsbury, Gillian (Millennium Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2001000810 A1 20010104, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US18152 20000630. PRIORITY: US 1999-345468 19990630; US 1999-454824 19991206; US 2000-503387 20000214.

AB The invention provides isolated cDNA mols. and polypeptide mols. that encode human and murine glycoprotein VI, a platelet membrane glycoprotein that is involved platelet-collagen interactions. The protein initially designated TANGO 268 represents the platelet-expressed collagen receptor glycoprotein VI (**GPVI**) based on the following evidence: (1) the glycosylated mol. wts. of TANGO 268 and **GPVI** are identical or similar; (2) both are recognized by anti-**GPVI antibodies** and bind to convulxin; (3) both are preferentially expressed in megakaryocytic cells; (4) both ar predicted to have a single N-glycosylation site; (5) the mol. mass of **GPVI** upon N- and O-linked glycosylation is .apprx.62 kDa, that of **GPVI**; (6) two Ig-like domains in TANGO 268 indicates interaction with FcR.gamma.; (7) the absence of a large intracytoplasmic tail suggests that this membrane-bound glycoprotein has no signaling role but assocs. with another member of the Ig family; and (8) TANGO 268 has a charged arginine residue in the transmembrane domain which is also predicted to be present in **GPVI**. The human gene for **GPVI** was mapped on radiation hybrid panels to the long arm of chromosome 19, in the region 19q13, syntenic to mouse chromosome 7. The invention also provides antisense nucleic acid mols., expression vectors contg. the nucleic acid mols. of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid mol. of the invention has been introduced or disrupted The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and **antibodies**. Diagnostic, screening and therapeutic methods utilizing compns. of the invention are also provided.

L21 ANSWER 5 OF 44 MEDLINE DUPLICATE 2
2001436540 Document Number: 21359356. PubMed ID: 11344165. A novel viper venom metalloproteinase, alborhagin, is an agonist at the platelet collagen receptor **GPVI**. Andrews R K; Gardiner E E; Asazuma N; Berlanga O; Tulasne D; Nieswandt B; Smith A I; Berndt M C; Watson S P. (Hazel and Pip Appel Vascular Biology Laboratory and the Peptide Biology Laboratory, Baker Medical Research Institute, Melbourne 8008, Australia.. rkandrews@hotmail.com) . JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jul 27) 276 (30) 28092-7. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The interaction of platelet membrane glycoprotein VI (**GPVI**) with collagen can initiate (patho)physiological thrombus formation. The viper venom C-type lectin family proteins convulxin and alboaggregin-A activate platelets by interacting with **GPVI**. In this study, we isolated from white-lipped tree viper (Trimeresurus albolabris) venom, alborhagin, which is functionally related to convulxin because it activates platelets but is structurally different and related to venom metalloproteinases. Alborhagin-induced platelet aggregation (EC50, <7.5 microg/ml) was inhibitable by an anti-alphaIIbbeta3 **antibody**, CRC64, and the Src family kinase inhibitor PP1, suggesting that alborhagin activates platelets, leading to alphaIIbbeta3-dependent aggregation. Additional evidence suggested that, like convulxin, alborhagin activated platelets by a mechanism involving **GPVI**. First, alborhagin- and convulxin-treated platelets showed a similar tyrosine phosphorylation pattern, including a similar level of phospholipase Cgamma2 phosphorylation. Second, alborhagin induced **GPVI**-dependent responses in **GPVI**-transfected K562 and Jurkat cells. Third, alborhagin-dependent aggregation of mouse platelets was inhibited by the anti-**GPVI** monoclonal **antibody** JAQ1. Alborhagin had minimal effect on convulxin binding to **GPVI**-expressing cells, indicating that these venom proteins may recognize distinct binding sites. Characterization of alborhagin as a **GPVI** agonist that is structurally distinct from convulxin demonstrates the versatility of snake venom toxins and provides a novel probe for **GPVI**-dependent platelet activation.

L21 ANSWER 6 OF 44 MEDLINE DUPLICATE 3
2001398776 Document Number: 21326192. PubMed ID: 11352922. Rhodocytin (aggretin) activates platelets lacking alpha(2)beta(1) integrin, glycoprotein VI, and the ligand-binding domain of glycoprotein Ibalpha. Bergmeier W; Bouvard D; Eble J A; Mokhtari-Nejad R; Schulte V; Zirngibl H; Brakebusch C; Fassler R; Nieswandt B. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, Arrenbergerstr. 20, Haus 10, 42117 Wuppertal, Germany.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jul 6) 276 (27) 25121-6. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Although alpha(2)beta(1) integrin (glycoprotein Ia/IIa) has been established as a platelet collagen receptor, its role in collagen-induced platelet activation has been controversial. Recently, it has been demonstrated that rhodocytin (also termed aggretin), a snake venom toxin purified from the venom of Calloselasma rhodostoma, induces platelet activation that can be blocked by monoclonal **antibodies** against alpha(2)beta(1) integrin. This finding suggested that clustering of alpha(2)beta(1) integrin by rhodocytin is sufficient to induce platelet activation and led to the hypothesis that collagen may activate platelets by a similar mechanism. In contrast to these findings, we provided evidence that rhodocytin does not bind to alpha(2)beta(1) integrin. Here we show that the Cre/loxP-mediated loss of beta(1) integrin on mouse platelets has no effect on rhodocytin-induced platelet activation, excluding an essential role of alpha(2)beta(1) integrin in this process. Furthermore, proteolytic cleavage of the 45-kDa N-terminal domain of glycoprotein (GP) Ibalpha either on normal or on beta(1)-null platelets had no significant effect on rhodocytin-induced platelet activation. Moreover, mouse platelets lacking both alpha(2)beta(1) integrin and the

activating collagen receptor **GPVI** responded normally to rhodocytin. Finally, even after additional proteolytic removal of the 45-kDa N-terminal domain of GPIbalpha rhodocytin induced aggregation of these platelets. These results demonstrate that rhodocytin induces platelet activation by mechanisms that are fundamentally different from those induced by collagen.

L21 ANSWER 7 OF 44 MEDLINE DUPLICATE 4
2001350473 Document Number: 21293088. PubMed ID: 11287424. Aggretin, a heterodimeric C-type lectin from Calloselasma rhodostoma (malayan pit viper), stimulates platelets by binding to alpha 2beta 1 integrin and glycoprotein Ib, activating Syk and phospholipase Cgamma 2, but does not involve the glycoprotein VI/Fc receptor gamma chain collagen receptor. Navdaev A; Clemetson J M; Polgar J; Kehrel B E; Glauner M; Magnenat E; Wells T N; Clemetson K J. (Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-3012 Berne, Switzerland.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jun 15) 276 (24) 20882-9. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB Aggretin, a potent platelet activator, was isolated from Calloselasma rhodostoma venom, and 30-amino acid N-terminal sequences of both subunits were determined. Aggretin belongs to the heterodimeric snake C-type lectin family and is thought to activate platelets by binding to platelet glycoprotein alpha(2)beta(1). We now show that binding to glycoprotein (GP) Ib is also required. Aggretin-induced platelet activation was inhibited by a monoclonal **antibody** to GPIb as well as by **antibodies** to alpha(2)beta(1). Binding of both of these platelet receptors to aggretin was confirmed by affinity chromatography. No binding of other major platelet membrane glycoproteins, in particular **GPVI**, to aggretin was detected. Aggretin also activates platelets from Fc receptor gamma chain (Fcgamma)-deficient mice to a greater extent than those from normal control mice, showing that it does not use the **GPVI**/Fcgamma pathway. Platelets from Fcgamma-deficient mice expressed fibrinogen receptors normally in response to collagen, although they did not aggregate, indicating that these platelets may partly compensate via other receptors including alpha(2)beta(1) or GPIb for the lack of the Fcgamma pathway. Signaling by aggretin involves a dose-dependent lag phase followed by rapid tyrosine phosphorylation of a number of proteins. Among these are p72(SYK), p125(FAK), and PLCgamma2, whereas, in comparison with collagen and convulxin, the Fcgamma subunit neither is phosphorylated nor coprecipitates with p72(SYK). This supports an independent, GPIb- and integrin-based pathway for activation of p72(SYK) not involving the Fcgamma receptor.

L21 ANSWER 8 OF 44 MEDLINE DUPLICATE 5
2001370835 Document Number: 21226781. PubMed ID: 11278467. Expression and function of the collagen receptor **GPVI** during megakaryocyte maturation. Lagrue-Lak-Hal A H; Debili N; Kingbury G; Lecut C; Le Couedic J P; Villeval J L; Jandrot-Perrus M; Vainchenker W. (INSERM E9907, Faculte Xavier Bichat, 75870 Paris Cedex 18, Paris, France.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 May 4) 276 (18) 15316-25. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States Language: English.
AB In this report, the expression and function of the platelet collagen receptor glycoprotein VI (**GPVI**) were studied in human megakaryocytes during differentiation and maturation of mobilized blood and cord blood derived CD34(+) cells. By flow cytometry, using an anti-**GPVI** monoclonal **antibody** or convulxin, a **GPVI**-specific ligand, **GPVI** was detected only on CD41(+) cells including some CD41(+)/CD34(+) cells, suggesting expression at a stage of differentiation similar to CD41. These results were confirmed at the mRNA level using reverse transcription-polymerase chain reaction. **GPVI** expression was low during megakaryocytic differentiation but increased in the more mature megakaryocytes (CD41(high)). As in platelets, megakaryocyte **GPVI** associates with the Fc receptor gamma chain (FcRgamma). The FcR gamma chain was detected at the RNA and protein level at all stages of megakaryocyte maturation preceding the expression of

GPVI. The other collagen receptor, alpha(2)beta(1) integrin (CD49b/CD29), had a pattern of expression similar to **GPVI**. Megakaryocytic **GPVI** was recognized as a 55-kDa protein by immunoblotting and ligand blotting, and thus it presented a slightly lower apparent molecular mass than platelet **GPVI** (58 kDa). Megakaryocytes began to adhere to immobilized convulxin via **GPVI** after only 8-10 days of culture, at a time when megakaryocytes were maturing. At this stage of maturation, they also adhered to immobilized collagen by alpha(2)beta(1) integrin-dependent and -independent mechanisms Convulxin induced a very similar pattern of protein tyrosine phosphorylation in megakaryocytes and platelets including Syk, FcRgamma, and PLC(gamma)2. Our results showed that **GPVI** is expressed early during megakaryocytic differentiation but functionally allows megakaryocyte adherence to collagen only at late stages of differentiation when its expression increases.

L21 ANSWER 9 OF 44 MEDLINE DUPLICATE 6
2001335037 Document Number: 21282622. PubMed ID: 11389023. Evidence for cross-talk between glycoprotein VI and Gi-coupled receptors during collagen-induced platelet aggregation. Nieswandt B; Bergmeier W; Eckly A; Schulte V; Ohlmann P; Cazenave J P; Zirngibl H; Offermanns S; Gachet C. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, Arrenbergerstrasse 20, 42117 Wuppertal, Germany.. nieswand@klinikum-wuppertal.de) . BLOOD, (2001 Jun 15) 97 (12) 3829-35. Journal code: A8G; 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Collagen-induced platelet aggregation is a complex process and involves synergistic action of integrins, immunoglobulin (Ig)-like receptors, G-protein-coupled receptors and their ligands, most importantly collagen itself, thromboxane A(2) (TXA(2)), and adenosine diphosphate (ADP). The precise role of each of these receptor systems in the overall processes of activation and aggregation, however, is still poorly defined. Among the collagen receptors expressed on platelets, glycoprotein (GP) VI has been identified to play a crucial role in collagen-induced activation. **GPVI** is associated with the FcRgamma chain, which serves as the signal transducing unit of the receptor complex. It is well known that clustering of **GPVI** by highly specific agonists results in platelet activation and irreversible aggregation, but it is unclear whether collagen has the same effect on the receptor. This study shows that platelets from Galphaq-deficient mice, despite their severely impaired response to collagen, normally aggregate on clustering of **GPVI**, suggesting this not to be the principal mechanism by which collagen activates platelets. On the other hand, dimerization of **GPVI** by a monoclonal **antibody** (JAQ1), which by itself did not induce aggregation, provided a sufficient stimulus to potentiate platelet responses to Gi-coupled, but not Gq-coupled, agonists. The combination of JAQ1 and adrenaline or ADP, but not serotonin, resulted in alpha(IIb)beta(3)-dependent aggregation that occurred without intracellular calcium mobilization and shape change in the absence of Galphaq or the P2Y(1) receptor. Together, these results provide evidence for a cross-talk between (dimerized) **GPVI** and Gi-coupled receptors during collagen-induced platelet aggregation. (Blood. 2001;97:3829-3835)

L21 ANSWER 10 OF 44 MEDLINE
2001272329 Document Number: 21231159. PubMed ID: 11331578. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. Nieswandt B; Brakebusch C; Bergmeier W; Schulte V; Bouvard D; Mokhtari-Nejad R; Lindhout T; Heemskerk J W; Zirngibl H; Fassler R. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, 42117 Wuppertal, Germany.. nieswand@klinikum-wuppertal.de) . EMBO JOURNAL, (2001 May 1) 20 (9) 2120-30. Journal code: EMB; 8208664. ISSN: 0261-4189. Pub. country: England: United Kingdom. Language: English.

AB Platelet adhesion on and activation by components of the extracellular matrix are crucial to arrest post-traumatic bleeding, but can also harm

tissue by occluding diseased vessels. Integrin alpha2beta1 is thought to be essential for platelet adhesion to subendothelial collagens, facilitating subsequent interactions with the activating platelet collagen receptor, glycoprotein VI (**GPVI**). Here we show that Cre/loxP-mediated loss of beta1 integrin on platelets has no significant effect on the bleeding time in mice. Aggregation of beta1-null platelets to native fibrillar collagen is delayed, but not reduced, whereas aggregation to enzymatically digested soluble collagen is abolished. Furthermore, beta1-null platelets adhere to fibrillar, but not soluble collagen under static as well as low (150 s(-1)) and high (1000 s(-1)) shear flow conditions, probably through binding of alphaIIbbeta3 to von Willebrand factor. On the other hand, we show that platelets lacking **GPVI** can not activate integrins and consequently fail to adhere to and aggregate on fibrillar as well as soluble collagen. These data show that **GPVI** plays the central role in platelet-collagen interactions by activating different adhesive receptors, including alpha2beta1 integrin, which strengthens adhesion without being essential.

L21 ANSWER 11 OF 44 MEDLINE DUPLICATE 7

2002088264 Document Number: 21591259. PubMed ID: 11816718. Bilinexin, a snake C-type lectin from Agkistrodon bilineatus venom agglutinates platelets via GPIb and alpha2beta1. Du X Y; Navdaev A; Clemetson J M; Magnenat E; Wells T N; Clemetson K J. (Theodor Kocher Institute, University of Berne, Switzerland.) THROMBOSIS AND HAEMOSTASIS, (2001 Nov) 86 (5) 1277-83. Journal code: 7608063. ISSN: 0340-6245. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB A new snake protein, named bilinexin, has been purified from Agkistrodon bilineatus venom by ion-exchange chromatography and gel filtration chromatography. Under non-reducing conditions it has a mass of 110 kDa protein on SDS-PAGE. On reduction, it can be separated into five subunits with masses in the range 13-25 kDa. The N-terminal sequences of these subunits are very similar to those of convulxin or the alboaggregins, identifying bilinexin as a new member of the snake C-type lectin family, unusual in having multiple subunits. Bilinexin agglutinates fixed platelets. washed platelets and platelet rich plasma (PRP) without obvious activation (shape change) as confirmed by light microscope examination. Both inhibitory and binding studies indicate that **antibodies** against alpha2beta1 inhibit not only platelet agglutination induced by bilinexin, but also bilinexin binding to platelets. VM16d, a monoclonal anti-GPIbalpha **antibody**, completely inhibits platelet agglutination induced by bilinexin, and polyclonal **antibodies** against GPIbalpha prevent its binding to platelets. However, neither convulxin, polyclonal anti-**GPVI antibodies**, nor GPIIb/IIIa inhibitors affect its binding to and agglutination of platelets. Bilinexin neither activates GPIIb/IIIa integrin on platelets nor induces tyrosine phosphorylation of platelet proteins, nor increases intracellular Ca2+ in platelets. Like alboaggregin B, bilinexin agglutinates platelets, which makes it a good tool to investigate the differences in mechanism between snake C-type lectins causing platelet agglutination and those that induce full activation.

L21 ANSWER 12 OF 44 MEDLINE DUPLICATE 8

2001446261 Document Number: 21384761. PubMed ID: 11493449. Platelet glycoprotein V binds to collagen and participates in platelet adhesion and aggregation. Moog S; Mangin P; Lenain N; Strassel C; Ravanat C; Schuhler S; Freund M; Santer M; Kahn M; Nieswandt B; Gachet C; Cazenave J P; Lanza F. (INSERM U.311, Etablissement Francais du Sang-Alsace, Strasbourg, France.) BLOOD, (2001 Aug 15) 98 (4) 1038-46. Journal code: A8G; 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Glycoprotein V (GPV) is a subunit of the platelet GPIb-V-IX receptor for von Willebrand factor and thrombin. GPV is cleaved from the platelet surface during activation by thrombin, but its role in hemostasis is still unknown. It is reported that GPV knockout mice had a decreased tendency to form arterial occluding thrombi in an intravital thrombosis model and abnormal platelet interaction with the subendothelium. In vitro,

GPV-deficient platelets exhibited defective adhesion to a collagen type I-coated surface under flow or static conditions. Aggregation studies demonstrated a decreased response of the GPV-deficient platelets to collagen, reflected by an increased lag phase and reduced amplitude of aggregation. Responses to adenosine diphosphate, arachidonic acid, and the thromboxane analog U46619 were normal but were enhanced to low thrombin concentrations. The defect of GPV null platelets made them more sensitive to inhibition by the anti-**GPVI** monoclonal **antibody** (mAb) JAQ1, and this was also the case in aspirin- or apyrase-treated platelets. Moreover, an mAb (V.3) against the extracellular domain of human GPV selectively inhibited collagen-induced aggregation in human or rat platelets. V.3 injected in rats as a bolus decreased the ex vivo collagen aggregation response without affecting the platelet count. Finally, surface plasmon resonance studies demonstrated binding of recombinant soluble GPV on a collagen-coupled matrix. In conclusion, GPV binds to collagen and appears to be required for normal platelet responses to this agonist. (Blood. 2001;98:1038-1046)

L21 ANSWER 13 OF 44 MEDLINE DUPLICATE 9

2001258216 Document Number: 21102921. PubMed ID: 11181698. Long-term antithrombotic protection by in vivo depletion of platelet glycoprotein VI in mice. Nieswandt B; Schulte V; Bergmeier W; Mokhtari-Nejad R; Rackebrandt K; Cazenave J P; Ohlmann P; Gachet C; Zirngibl H. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, 42117 Wuppertal, Germany.. nieswand@klinikum-wuppertal.de) . JOURNAL OF EXPERIMENTAL MEDICINE, (2001 Feb 19) 193 (4) 459-69. Journal code: I2V; 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque and activation of platelets on the subendothelial layers in the disrupted plaque. The extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer; therefore, a selective inhibition of the collagen activation pathway in platelets may provide strong antithrombotic protection while preserving other platelet functions. Here we demonstrate that treatment of mice with a monoclonal **antibody** against the activating platelet collagen receptor glycoprotein VI (**GPVI**; JAQ1) results in specific depletion of the receptor from circulating platelets and abolished responses of these cells to collagen and collagen-related peptides (CRPs). JAQ1-treated mice were completely protected for at least 2 wk against lethal thromboembolism induced by infusion of a mixture of collagen (0.8 mg/kg) and epinephrine (60 microg/ml). The tail bleeding times in JAQ1-treated mice were only moderately increased compared with control mice probably because the treatment did not affect platelet activation by other agonists such as adenosine diphosphate or phorbol myristate acetate. These results suggest that **GPVI** might become a target for long-term prophylaxis of ischemic cardiovascular diseases and provide the first evidence that it is possible to specifically deplete an activating glycoprotein receptor from circulating platelets in vivo.

L21 ANSWER 14 OF 44 MEDLINE DUPLICATE 10

2001112665 Document Number: 20576376. PubMed ID: 11036078. Evidence for two distinct epitopes within collagen for activation of murine platelets. Schulte V; Snell D; Bergmeier W; Zirngibl H; Watson S P; Nieswandt B. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, 42117 Wuppertal, Germany.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jan 5) 276 (1) 364-8. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB It has recently been shown that the monoclonal **antibody** JAQ1 to murine glycoprotein VI (**GPVI**) can cause aggregation of mouse platelets upon **antibody** cross-linking and that collagen-induced platelet aggregation can be inhibited by preincubation of platelets with JAQ1 in the absence of cross-linking (Nieswandt, B., Bergmeier, W., Schulte, V., Rackebrandt, K., Gessner, J. E., and Zirngibl, H. (2000) J. Biol. Chem. 275, 23998-24002). In the present study, we have shown that cross-linking of **GPVI** by JAQ1 results in tyrosine

phosphorylation of the same profile of proteins as that induced by collagen, including the Fc receptor (FcR) gamma-chain, Syk, LAT, SLP-76, and phospholipase C gamma 2. In contrast, platelet aggregation and tyrosine phosphorylation of these proteins were inhibited when mouse platelets were preincubated with JAQ1 in the absence of cross-linking and were subsequently stimulated with a collagen-related peptide (CRP) that is specific for **GPVI** and low concentrations of collagen. However, at higher concentrations of collagen, but not CRP, aggregation of platelets and tyrosine phosphorylation of the above proteins (except for the adapter LAT) is re-established despite the presence of JAQ1. These observations suggest that a second activatory binding site, which is distinct from the CRP binding site on **GPVI** on mouse platelets, is occupied in the presence of high concentrations of collagen. Although this could be a second site on **GPVI** that is activated by a novel motif within the collagen molecule, the absence of LAT phosphorylation in response to collagen in the presence of JAQ1 suggests that this is more likely to be caused by activation of a second receptor that is also coupled to the FcR gamma-chain. The possibility that this response is mediated by a receptor that is not coupled to FcR gamma-chain is excluded on the grounds that aggregation is absent in platelets from FcR gamma-chain-deficient mice.

L21 ANSWER 15 OF 44 MEDLINE DUPLICATE 11
2001388659 Document Number: 21335840. PubMed ID: 11443641.
Antibody against platelet membrane glycoprotein VI in a patient with systemic lupus erythematosus. Takahashi H; Moroi M. (Department of Internal Medicine, Niigata Prefectural Kamo Hospital, Kamo, Niigata, Japan.) AMERICAN JOURNAL OF HEMATOLOGY, (2001 Aug) 67 (4) 262-7. Journal code: 3H4; 7610369. ISSN: 0361-8609. Pub. country: United States. Language: English.

AB Platelet-collagen interaction is important in primary hemostasis and collagen receptors on the platelet surface include membrane glycoprotein (GP) Ia/IIa and VI. Platelets from a 47-year-old woman with systemic lupus erythematosus (SLE) and a mild bleeding symptom showed a defective collagen-induced aggregation and an impaired adhesion to collagen surface. The patient's platelets had a markedly decreased content of **GPVI** . The patient had an **antibody** against **GPVI** in serum and the patient's plasma induced aggregation and release reaction of normal platelets. These findings indicate that **GPVI** is an important receptor for collagen on the platelet surface, and that anti-**GPVI antibody** activates the platelets, resulting in aggregation. This is the first documented case of SLE who acquired a platelet-aggregating anti-**GPVI antibody**.

L21 ANSWER 16 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:129535 Document No.: PREV200200129535. The platelet collagen receptor glycoprotein VI (**GPVI**) signals through lipid rafts in a Fc Rgamma-dependent manner. Locke, Darren (1); Chen, Hong (1); Liu, Chang-Dong (1); Kahn, Mark L. (1). (1) Molecular Cardiology, University of Pennsylvania, Philadelphia, PA USA. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 25a. http://www.bloodjournal.org/. print. Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001 ISSN: 0006-4971. Language: English.

AB The platelet collagen receptor **GPVI** signals through the immunoreceptor tyrosine activation motif (ITAM) of its co-receptor Fc Rgamma using many of the same downstream signaling proteins as T cell, B cell and Fc receptors. Signaling by these immune receptors is believed to proceed from receptor clustering to ITAM tyrosine phosphorylation by the src family kinases Fyn and Lyn and subsequent activation of the tyrosine kinases Syk or ZAP-70. Activation of immune receptors results in receptor movement to cholesterol-rich areas of the cell membrane known as lipid rafts that are enriched in Fyn, Lyn and the transmembrane adaptor protein LAT and are defined by their resistance to solubilization by non-ionic detergents. To determine whether activation of **GPVI** results in

receptor movement to lipid rafts we expressed **GPVI** in RBL-2H3 cells, a mast cell line which expresses abundant Fc Rgamma but no known collagen receptors. Activation of **GPVI** with the agonist convulxin resulted in a rapid, transient movement of **GPVI** receptors to lipid rafts, a response which was also seen with activation of endogenous Fc epsilon receptors which also couple to Fc Rgamma. The mechanism by which immune receptor activation results in receptor movement to lipid rafts is unknown. To determine the contribution of Fc Rgamma for **GPVI** movement to lipid rafts we examined the behavior of **GPVI** R272L, a previously characterized mutant **GPVI** receptor in which a single amino acid substitution results in loss of Fc Rgamma coupling and intracellular signaling despite normal surface expression. **GPVI** R272L binds CVX but does not move to lipid rafts following ligand binding, suggesting that **GPVI** receptor movement to lipid rafts is mediated by the Fc Rgamma chain. The role of lipid rafts in platelet signaling by **GPVI** and other receptors has not been defined. Using a novel anti-**GPVI** monoclonal **antibody**, HY101, we have isolated lipid rafts from human platelets and shown that, like **GPVI**-expressing RBL-2H3 cells, platelet stimulation of **GPVI** by convulxin results in the transient movement of **GPVI** to lipid rafts. Our results demonstrate that (1) during **GPVI** signaling the receptor moves to lipid rafts in both RBL-2H3 cells and in human platelets, and (2) **GPVI** movement to lipid rafts following ligand binding is driven by associated Fc Rgamma chain and is not a simple consequence of ligand-induced receptor clustering. Studies are presently underway to determine whether **GPVI**-Fc Rgamma movement to lipid rafts is required for ITAM phosphorylation or vice-versa and to better define the role of lipid rafts for signaling by collagen in human platelets.

L21 ANSWER 17 OF 44 MEDLINE DUPLICATE 12
2000420885 Document Number: 20379043. PubMed ID: 10825177. Expression and function of the mouse collagen receptor glycoprotein VI is strictly dependent on its association with the FcRgamma chain. Nieswandt B; Bergmeier W; Schulte V; Rackebrandt K; Gessner J E; Zirngibl H. (Department of Molecular Oncology, General Surgery, University of Witten-Herdecke, 42283 Wuppertal, Germany.. niesand@klinikum-wuppertal.de) . JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Aug 4) 275 (31) 23998-4002. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Platelet glycoprotein (GP) VI has been proposed as the major collagen receptor for activation of human platelets. Human **GPVI** belongs to the immunoglobulin superfamily and is noncovalently associated with the FcRgamma chain that is involved in signaling through the receptor. In mice, similar mechanisms seem to exist as platelets from FcRgamma chain-deficient mice do not aggregate in response to collagen. However, the activating collagen receptor on mouse platelets has not been definitively identified. In the current study we examined the function and in vivo expression of **GPVI** in control and FcRgamma chain-deficient mice with the first monoclonal **antibody** against **GPVI** (JAQ1). On wild type platelets, JAQ1 inhibited platelet aggregation induced by collagen but not PMA or thrombin. Cross-linking of bound JAQ1, on the other hand, induced aggregation of wild type but not FcRgamma chain-deficient platelets. JAQ1 stained platelets and megakaryocytes from wild type but not FcRgamma chain-deficient mice. Furthermore, JAQ1 recognized **GPVI** (approximately 60 kDa) in immunoprecipitation and Western blot experiments with wild type but not FcRgamma chain-deficient platelets. These results strongly suggest that **GPVI** is the collagen receptor responsible for platelet activation in mice and demonstrate that the association with the FcRgamma chain is critical for its expression and function.

L21 ANSWER 18 OF 44 MEDLINE DUPLICATE 13
2000193634 Document Number: 20193634. PubMed ID: 10727949. Evidence against a direct role of the integrin alpha2beta1 in collagen-induced

tyrosine phosphorylation in human platelets. Hers I; Berlanga O; Tiekstra M J; Kamiguti A S; Theakston R D; Watson S P. (Department of Pharmacology, University of Oxford, UK.) EUROPEAN JOURNAL OF BIOCHEMISTRY, (2000 Apr) 267 (7) 2088-97. Journal code: EMZ; 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB In the present study we have investigated whether the collagen receptor alpha2beta1 (GPIa-IIa; GP, glycoprotein) regulates protein tyrosine phosphorylation in platelets directly through activation of tyrosine kinases or indirectly through modification of the response to **GPVI** . The interaction of collagen with alpha2beta1 was inhibited in two distinct ways, using the metalloprotease jararhagin, which cleaves the beta1 subunit, or the **antibody** P1E6 which competes with binding of collagen to the integrin. The two inhibitors caused a shift to the right in the collagen concentration response curves for protein tyrosine phosphorylation and platelet activation consistent with a causal relationship between the two events. There was no change in the overall pattern of tyrosine phosphorylation in response to high concentrations of collagen in the presence of alpha2beta1 blockade demonstrating that the integrin is not required for this event. In contrast, jararhagin and P1E6 had a small, almost negligible inhibitory effect against responses to the **GPVI**-selective agonist collagen-related peptide (CRP) and the G protein-coupled receptor agonist thrombin. Crosslinking of alpha2beta1 in solution or by adhesion to a monolayer using a variety of **antibodies** to either subunit of the integrin did not induce detectable protein tyrosine phosphorylation in whole cell lysates. The snake venom toxin trimucytin-stimulated a similar pattern of tyrosine phosphorylation to that induced by crosslinking of **GPVI** which was maintained in the presence of jararhagin. Trimucytin may therefore induce activation via **GPVI** rather than alpha2beta1 as previously thought. These observations show that the integrin alpha2beta1 is not required for regulation of tyrosine phosphorylation by collagen.

L21 ANSWER 19 OF 44 MEDLINE DUPLICATE 14

2000132875 Document Number: 20132875. PubMed ID: 10666318. Distinct contributions of glycoprotein VI and alpha(2)beta(1) integrin to the induction of platelet protein tyrosine phosphorylation and aggregation. Kamiguti A S; Theakston R D; Watson S P; Bon C; Laing G D; Zuzel M. (Department of Haematology, Royal Liverpool Hospital, Liverpool, United Kingdom.. aurakami@liverpool.ac.uk) . ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (2000 Feb 15) 374 (2) 356-62. Journal code: 6SK; 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB Platelet activation by collagen depends principally on two receptors, alpha(2)beta(1) integrin (GPIa-IIa) and **GPVI**. During this activation, the nonreceptor protein tyrosine kinase pp72(syk) is rapidly phosphorylated, but the precise contribution of alpha(2)beta(1) integrin and **GPVI** to signaling for this phosphorylation is not clear. We have recently found that proteolysis of platelet alpha(2)beta(1) integrin by the snake venom metalloproteinase, jararhagin, results in inhibition of collagen-induced platelet aggregation and pp72(syk) phosphorylation. In order to verify whether the treatment of platelets with jararhagin had any effect on **GPVI** signaling, in this study we stimulated platelets treated with either jararhagin or anti-alpha(2)beta(1) **antibody** with two **GPVI** agonists, an **antibody** to **GPVI** and convulxin. Platelet shape change and phosphorylation of pp72(syk) by both **GPVI** agonists was preserved, as was the structure and function of **GPVI** shown by (125)I-labeled convulxin binding to immunoprecipitated **GPVI** from jararhagin-treated platelets. In contrast, defective platelet aggregation in response to **GPVI** agonists occurred in both jararhagin-treated and alpha(2)beta(1)-blocked platelets. This apparent cosignaling role of alpha(2)beta(1) integrin for platelet aggregation suggests the possibility of a topographical association of this integrin with **GPVI**. We found that both platelet alpha(2)beta(1) integrin and **GPVI** coimmunoprecipitated with alpha(IIb)beta(3) integrin. Since platelet aggregation requires activation of alpha(IIb)beta(3) integrin, defective aggregation in the

absence of alpha(2)beta(1) suggests that this receptor may provide a signaling link between **GPVI** and alpha(IIb)beta(3). Our study therefore demonstrates that platelet signaling leading to pp72(syk) phosphorylation initiated with **GPVI** engagement by either convulxin or **GPVI antibody** does not depend on alpha(2)beta(1) integrin. However, alpha(IIb)beta(3) integrin may, in this model, require functional alpha(2)beta(1) integrin for its activation.

L21 ANSWER 20 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:393601 The Genuine Article (R) Number: 316BC. Cloning and expression of the platelet-specific collagen receptor glycoprotein VI. Miura Y; Ohnuma M; Jung S M; Moroi M (Reprint). KURUME UNIV, INST LIFE SCI, DEPT PROT BIOCHEM, 2432-3 AIKAWA MACHI, KURUME, FUKUOKA 8390861, JAPAN (Reprint); KURUME UNIV, INST LIFE SCI, DEPT PROT BIOCHEM, KURUME, FUKUOKA 8390861, JAPAN. THROMBOSIS RESEARCH (15 MAY 2000) Vol. 98, No. 4, pp. 301-309. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0049-3848. Pub. country: JAPAN. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Platelet glycoprotein VI (GP VI) was purified from platelet membranes and its internal amino acid sequences were determined. The cloned cDNA of GP VI indicates an open reading frame coding for 20 amino acid signal sequences and a mature protein of 319 amino acids. Its extracellular region has two Ig-like domains and a mucin-like, Ser/Thr-rich region, suggesting that GP VI is a member of the paired Ig-like receptor family. GP VI-transfected cells contained convulxin-(reactive) and **antibody** against recombinant GP VI-reactive protein bands that migrated at the same position as platelet GP VI in SDS/PAGE-electroblotting. These data indicate that the protein deduced from the cloned cDNA corresponds to platelet GP VI.

L21 ANSWER 21 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:311599 Document No.: PREV200100311599. Long-term antithrombotic protection by irreversible inactivation of platelet glycoprotein VI in mice. Nieswandt, Bernhard (1); Schulte, Valerie (1); Bergmeier, Wolfgang (1); Mokhtari-Nejad, Rabee (1); Cazenave, Jean P.; Gachet, Christian; Zirngibl, Hubert (1). (1) Molecular Oncology, Witten/Herdecke University, Wuppertal Germany. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 269a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology. ISSN: 0006-4971. Language: English. Summary Language: English.
AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque followed by deposition and activation of platelets on the subendothelial layers in the disrupted plaque. Because the extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer, a selective inhibition of the collagen activation pathway in platelets may provide strong antithrombotic protection while preserving other platelet functions. Growing evidence suggests that platelet glycoprotein (GP) VI is the major collagen receptor for platelet activation making this receptor a good candidate for such a specific inhibition. In the current study, we have investigated the antithrombotic effects of the first monoclonal **antibody** (mAb) against mouse **GPVI** (JAQ1, Nieswandt et al; 2000, J Biol Chem, 275(31):23998-24002). Injection of 100 mug JAQ1 only had mild and transient effects on platelet counts with a maximum drop of approximately 34 +- 7.4 % on day 1 and a return to normal after 2-3 days. JAQ1 pretreated mice were completely protected against lethal thromboembolism induced by infusion of a mixture of collagen (0 8 mg/kg) and epinephrine (60 mug/kg) for at least two weeks (100% survivors on days 3, 7, and 14 after mAb injection, n=8 per group, 5% survivors in the control group, n=20). Aggregometric and flow cytometric studies demonstrated that platelets from JAQ1 treated mice were completely resistant against

activation with high concentrations of collagen (up to 50 mug/ml) and collagen related peptides (up to 100 mug/ml) ex vivo on days 3, 7, and 14. In JAQ1 treated mice, **GPVI** was not detectable in a Western blot analysis of platelet lysates for minimally two weeks, suggesting irreversible inactivation (or degradation) of the receptor on circulating platelets. In contrast to collagen, other agonists, such as ADP or platelet aggregating agents, such as PMA induced normal activation and aggregation of these platelets. Consequently, the tail bleeding times were only moderately increased in anti-**GPVI** treated mice compared to control mice on day 3, 7, and 14. These results establish **GPVI** as an attractive target for long-term antithrombotic therapy.

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1999:736770 Document No. 131:350263 Chimeric proteins containing IgG Fc fragments which do not trigger complement mediated lysis. Armour, Kathryn Lesley; Clark, Michael Ronald; Williamson, Lorna McLeod (Cambridge University Technical Services Limited, UK). PCT Int. Appl. WO 9958572 A1 19991118, 81 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1441 19990507. PRIORITY: GB 1998-9951 19980508.

AB The authors disclose recombinant polypeptides comprising: (i) a binding domain capable of binding a target mol., and (ii) an effector domain having an amino acid sequence substantially homologous to all or part of a const. domain of a human Ig heavy chain. These chimeric proteins are capable of binding the target mol. without triggering significant complement dependent lysis, or cell mediated destruction of the target and, via the effector domain, remain capable of specifically binding FcRn and/or Fc.gamma.RIIb. These effector domains are derived from two or more human Ig heavy chain CH2 domains. The binding domain of the chimeric proteins may be derived from **antibodies**, enzymes, hormones, receptors, and cytokines etc.

L21 ANSWER 23 OF 44 MEDLINE DUPLICATE 15

1999436101 Document Number: 99436101. PubMed ID: 10506151. The platelet collagen receptor glycoprotein VI is a member of the immunoglobulin superfamily closely related to FcalphaR and the natural killer receptors. Clemetson J M; Polgar J; Magnenat E; Wells T N; Clemetson K J (Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-3012 Berne, Switzerland.. clemetson@tki.unibe.ch) . JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Oct 8) 274 (41) 29019-24. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB We have cloned the platelet collagen receptor glycoprotein (GP) VI from a human bone marrow cDNA library using rapid amplification of cDNA ends with platelet mRNA to complete the 5' end sequence. **GPVI** was isolated from platelets using affinity chromatography on the snake C-type lectin, convulxin, as a critical step. Internal peptide sequences were obtained, and degenerate primers were designed to amplify a fragment of the **GPVI** cDNA, which was then used as a probe to screen the library. Purified **GPVI**, as well as Fab fragments of polyclonal **antibodies** made against the receptor, inhibited collagen-induced platelet aggregation. The **GPVI** receptor cDNA has an open reading frame of 1017 base pairs coding for a protein of 339 amino acids including a putative 23-amino acid signal sequence and a 19-amino acid transmembrane domain between residues 247 and 265. **GPVI** belongs to the immunoglobulin superfamily, and its sequence is closely related to FcalphaR and to the natural killer receptors. Its extracellular chain has two Ig-C2-like domains formed by disulfide bridges. An arginine residue is found in position 3 of the transmembrane portion, which should permit association with Fcgamma and its immunoreceptor tyrosine-based activation

motif via a salt bridge. With 51 amino acids, the cytoplasmic tail is relatively long and shows little homology to the C-terminal part of the other family members. The ability of the cloned **GPVI** cDNA to code for a functional platelet collagen receptor was demonstrated in the megakaryocytic cell line Dami. Dami cells transfected with **GPVI** cDNA mobilized intracellular Ca(2+) in response to collagen, unlike the nontransfected or mock transfected Dami cells, which do not respond to collagen.

L21 ANSWER 24 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
1999163586 EMBASE Function of glycoprotein VI and integrin .alpha.2.beta.1 in the procoagulant response of single, collagen-adherent platelets. Heemskerk J.W.M.; Siljander P.; Vuist W.M.; Breikers G.; Reutelingsperger C.P.M.; Barnes M.J.; Knight C.G.; Lassila R.; Farndale R.W.. Dr. J.W.M. Heemskerk, Department of Biochemistry, University of Maastricht, PO Box 616, 6200 MD Maastricht, Netherlands. JWM.Heemskerk@Bioch.UniMaas.nl. Thrombosis and Haemostasis 81/5 (782-792) 1999.
Refs: 47.
ISSN: 0340-6245. CODEN: THHADQ. Pub. Country: Germany. Language: English. Summary Language: English.
AB Various collagen-based materials were used to assess the structural requirements of collagen for inducing the procoagulant response of adhering platelets, as well as the collagen receptors involved. Crosslinked or monomeric collagen-related peptide (CRP), Gly-Cys-Hyp-(Gly-Pro-Hyp)10-Gly-Cys-Hyp-Gly was highly adhesive for platelets in a glycoprotein VI- (**GpVI**-)dependent manner. Adhesion was followed by a prolonged increase in cytosolic [Ca2+](i), formation of membrane blebs, exposure of phosphatidylserine (PS and generation of prothrombinase-stimulating activity. Fibrils of type-I collagen were less adhesive but, once adhered, many of the platelets presented a full procoagulant response. Monomeric type-I collagen was unable to support adhesion, unless Mg2+-dependent integrin .alpha.2.beta.1 interactions were facilitated by omission of Ca2+ ions. With all surfaces, however, post-addition of CaCl2 to adhering platelets resulted in a potent Ca2+-influx signal, followed by PS exposure and bleb formation. The procoagulant response elicited by binding to CRP was inhibited by anti-**GpVI** Fab fragments, but not by impeding integrin .alpha.2.beta.1-mediated events. With fibrillar collagen, it was inhibited by blocking either the **GpVI**- or integrin .alpha.2.beta.1 mediated interactions. This suggests that the triple-helical Gly-Pro-Hyp repeat in CRP and analogous sequences in fibrillar collagen stimulate the procoagulant response of adherent platelets by acting as ligands for **GpVI**. Influx of Ca2+ is required for this response, and adhesion via integrin .alpha.2.beta.1 serves to potentiate the signaling effects of **GpVI**.

L21 ANSWER 25 OF 44 MEDLINE DUPLICATE 16
1999167348 Document Number: 99167348. PubMed ID: 10066433. Signal transduction pathways mediated by glycoprotein Ia/IIa in human platelets: comparison with those of glycoprotein VI. Inoue K; Ozaki Y; Satoh K; Wu Y; Yatomi Y; Shin Y; Morita T. (Department of Clinical and Laboratory Medicine, Yamanashi Medical University, Shimokato 1110 Tamaho, Yamanashi, Nakakoma, 409-3898, Japan.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 Mar 5) 256 (1) 114-20. Journal code: 9Y8; 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB Human platelets were activated either by glycoprotein (GP) Ia/IIa agonist (rhodocytin) or by a **GPVI** agonist (collagen-related peptide, CRP), and the intracellular signal transduction pathways were compared in the presence of various inhibitors. Rhodocytin isolated from Calloselasma rhodostoma venom was verified as a GPIa/IIa agonist, based on the inhibitory effects of three mAbs directed against GPIa. Platelet activation mediated by GPIa/IIa led to overt platelet aggregation, elevation of intracellular Ca2+, and tyrosine phosphorylation of several proteins, similar to that of **GPVI**. p72(syk) and phospholipase Cgamma2 (PLCgamma2) tyrosine phosphorylation were also observed with

GPIa/IIa-mediated platelet aggregation, although they peaked slightly later than that of **GPVI**. In contrast to **GPVI**-mediated platelet activation, most of these phenomena induced by GPIa/IIa activation were markedly suppressed by acetylsalicylic acid (ASA) or cytochalasin D. These findings suggest that the requirements for thromboxane A2 (TXA2) production and actin polymerization, which are the characteristics of collagen-induced platelet activation, are derived from the GPIa/IIa-mediated signal transduction, but not from that of **GPVI**.

L21 ANSWER 26 OF 44 MEDLINE DUPLICATE 17
1998136126 Document Number: 98136126. PubMed ID: 9468482. Platelet adhesion to native type I collagen fibrils. Role of **GPVI** in divalent cation-dependent and -independent adhesion and thromboxane A2 generation. Nakamura T; Jamieson G A; Okuma M; Kambayashi J; Tandon N N. (Otsuka America Pharmaceutical Inc., Rockville, Maryland 20850, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Feb 20) 273 (8) 4338-44. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB Three glycoproteins (GPs), namely GPIa-IIa, **GPVI**, and GPIV, have been recently implicated in platelet-collagen adhesive interactions. We have employed **antibodies** to these GPs to investigate further their role in platelet adhesion to immobilized monomeric and polymeric fibrillar collagen under static conditions in the presence and the absence of Mg2+. In the presence of Mg2+, each **antibody** inhibited platelet adhesion to fibrillar collagen from 70 to 85%, especially during the early phase (<15 min), but the inhibitory effects diminished dramatically to 25% or less by 60 min. Combination of anti-**GPVI** with anti-GPIa-IIa **antibodies** completely inhibited platelet adhesion at 60 min. Anti-GPIV and anti-GPIa-IIa or anti-**GPVI** **antibodies** in combinations were more effective in inhibiting adhesion than was anti-GPIa-IIa or anti-**GPVI** alone. In the absence of Mg2+, anti-**GPVI** completely inhibited adhesion at 60 min, while anti-GPIV **antibody** inhibited adhesion by about 50% and minimal effects were seen with anti-GPIa-IIa, suggesting that GPIa-IIa does not play a significant role in the divalent cation-independent platelet adhesion to immobilized fibrillar collagen. Under either divalent cation-dependent or -independent conditions, platelets adhered to fibrillar collagen were able to secrete contents of both alpha-granules and dense granules and generate thromboxane A2 (TXA2), but platelets adhering to acid soluble monomeric collagen neither secreted their granular contents nor generated TXA2. Although anti-**GPVI** **antibodies** were not able to inhibit Mg2+-dependent adhesion, they completely inhibited TXA2 generation under both divalent cation-dependent and -independent conditions. With the other **antibodies**, TXA2 generation corresponded with the amount of adhesion observed. These results suggest that **GPVI** is directly associated with the TXA2 generating system during platelet-collagen interaction.

L21 ANSWER 27 OF 44 MEDLINE DUPLICATE 18
1998241425 Document Number: 98241425. PubMed ID: 9573018. Simple collagen-like peptides support platelet adhesion under static but not under flow conditions: interaction via alpha2 beta1 and von Willebrand factor with specific sequences in native collagen is a requirement to resist shear forces. Verkleij M W; Morton L F; Knight C G; de Groot P G; Barnes M J; Sixma J J. (Postgradual School of Biomembranes, Department of Haematology, University Hospital Utrecht, Utrecht, The Netherlands.) BLOOD, (1998 May 15) 91 (10) 3808-16. Journal code: A8G; 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.
AB The aim of this study was to define the need for specific collagen sequences and the role of their conformation in platelet adhesion to collagen under both static and flow conditions. We recently reported that simple triple-helical collagen-related peptides (CRPs), GCP*(GPP*)10GCP*G and GKP*(GPP*)10GKP*G (single-letter amino acid code, P* = hydroxyproline;

Morton et al, Biochem J 306:337, 1995) were potent stimulators of platelet activation and were able to support the adhesion of gel-filtered platelets examined under static conditions. The present study investigated whether these same peptides were able to support platelet adhesion under more physiologic conditions by examining static adhesion with platelet-rich plasma (PRP) and adhesion under flow conditions. In the static adhesion assay, we observed 20% surface coverage with platelet aggregates. In marked contrast, there was a total lack of adhesion under flow conditions examined at shear rates of 50 and 300 s-1. Thus, the interaction of platelets with the CRPs is a low-affinity interaction unable on its own to withstand shear forces. However, the addition of CRPs to whole blood, in the presence of 200 micromol/L D-arginyl-glycyl-L-aspartyl-L-tryptophan (dRGDW) to prevent platelet aggregation, caused an inhibition of about 50% of platelet adhesion to collagens I and III under flow. These results suggest that the collagen triple helix per se, as defined by these simple collagen sequences, plays an important contributory role in the overall process of adhesion to collagen under flow. The monoclonal **antibody** (MoAb) 176D7, directed against the alpha2 subunit of the integrin alpha2 beta1, was found to inhibit static platelet adhesion to monomeric but not fibrillar collagens I and III. However, under flow conditions, anti-alpha2 MoAbs (176D7 anf 6F1) inhibited adhesion to both monomeric and fibrillar collagens, indicating that alpha2 beta1 is essential for adhesion to collagen under flow, independent of collagen conformation, whether monomeric or polymeric. To obtain further insight into the nature of the different adhesive properties of CRPs and native collagen, we investigated the relative importance of von Willebrand factor (vWF) and the integrin alpha2 beta1 in platelet adhesion to collagen types I and III, using the same shear rate (300 s-1) as used when testing CRPs under flow conditions. Our results, together with recent data of others, support a two-step mechanism of platelet interaction with collagen under flow conditions. The first step involves adhesion via both the indirect interaction of platelet glycoprotein (GP) Ib with collagen mediated by vWF binding to specific vWF-recognition sites in collagen and the direct interaction between platelet alpha2 beta1 and specific alpha2 beta1-recognition sites in collagen. This suffices to hold platelets at the collagen surface. The second step occurs via another collagen receptor (thought to be **GPVI**) that binds to simple collagen sequences, required essentially to delineate the collagen triple helix. Recognition of the triple helix leads to strengthening of attachment and platelet activation.

L21 ANSWER 28 OF 44 MEDLINE DUPLICATE 19

1998336213 Document Number: 98336213. PubMed ID: 9670039. Physical and functional association of the Src family kinases Fyn and Lyn with the collagen receptor glycoprotein VI-Fc receptor gamma chain complex on human platelets. Ezumi Y; Shindoh K; Tsuji M; Takayama H. (Department of Hematology and Oncology, Clinical Sciences for Pathological Organs, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan.) JOURNAL OF EXPERIMENTAL MEDICINE, (1998 Jul 20) 188 (2) 267-76. Journal code: I2V; 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB We have previously shown that uncharacterized glycoprotein VI (**GPVI**), which is constitutively associated and coexpressed with Fc receptor gamma chain (FcRgamma) in human platelets, is essential for collagen-stimulated tyrosine phosphorylation of FcRgamma, Syk, and phospholipase Cgamma2 (PLCgamma2), leading to platelet activation. Here we investigated involvement of the Src family in the proximal signals through the **GPVI**-FcRgamma complex, using the snake venom convulxin from Crotalus durissus terrificus, which specifically recognizes **GPVI** and activates platelets through cross-linking **GPVI**. Convulxin-coupled beads precipitated the **GPVI**-FcRgamma complex from platelet lysates. Collagen and convulxin induced tyrosine phosphorylation of FcRgamma, Syk, and PLCgamma2 and recruited tyrosine-phosphorylated Syk to the **GPVI**-FcRgamma complex. Using coprecipitation methods with convulxin-coupled beads and

antibodies against FcRgamma and the Src family, we showed that Fyn and Lyn, but not Yes, Src, Fgr, Hck, and Lck, were physically associated with the **GPVI**-FcRgamma complex irrespective of stimulation. Furthermore, Fyn was rapidly activated by collagen or cross-linking **GPVI**. The Src family-specific inhibitor PP1 dose-dependently inhibited collagen- or convulxin-induced tyrosine phosphorylation of proteins including FcRgamma, Syk, and PLCgamma2, accompanied by a loss of aggregation and ATP release reaction. These results indicate that the Src family plays a critical role in platelet activation via the collagen receptor **GPVI**-FcRgamma complex.

L21 ANSWER 29 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 20
1998228355 EMBASE Convulxin-induced platelet adhesion and aggregation: Involvement of glycoproteins VI and IaIIa. Jandrot-Perrus M.; Lagrue A.H.; Leduc M.; Okuma M.; Bon C.. Dr. M. Jandrot-Perrus, Lab. Recherche Hemostase Thrombose, Faculte de Medecine Xavier Bichat, 75870 Paris Cedex 18, France. Platelets 9/3-4 (207-211) 1998.
Refs: 22.
ISSN: 0953-7104. CODEN: PLTEEF. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB The interaction of convulxin (Cvx), a 72-kDa glycoprotein isolated from the venom of Crotalus durissus terrificus with human platelets has been studied. Cvx at low concentrations (below 100 pM) induced platelet aggregation, dense body secretion and intracellular calcium mobilization which indicates that Cvx is a potent activator of human platelets. Cvx-induced platelet aggregation and secretion was inhibited by 6F1 an anti-integrin .alpha.2.beta.1 monoclonal **antibody** that was without effect on calcium mobilization. Anti-**GPVI** Fab fragments inhibited aggregation, secretion and calcium mobilization triggered by Cvx. In addition, immobilized Cvx was found to induce divalent cation-independent platelet adhesion in a static system. Platelet adhesion to Cvx was inhibited by anti-**GPVI** Fab fragments but not by anti-integrin .alpha.2.beta.1. Cvx was shown to bind to a 57,000 Dalton protein that was identified as **GPVI**. Altogether, these results indicate that **GPVI** behaves as a receptor for Cvx, while integrin .alpha.2.beta.1 could play a regulatory role in Cvx-induced platelet aggregation. Cvx and collagen interaction with platelets, thus appears to share some characteristics but to also have specific properties.

L21 ANSWER 30 OF 44 MEDLINE DUPLICATE 21
1998001677 Document Number: 98001677. PubMed ID: 9341142. Adhesion and activation of human platelets induced by convulxin involve glycoprotein VI and integrin alpha2beta1. Jandrot-Perrus M; Lagrue A H; Okuma M; Bon C. (Laboratoire de Recherche sur l'Hemostase et la Thrombose, Faculte de Medecine Xavier Bichat, BP 416, 75870 Paris Cedex 18, France.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Oct 24) 272 (43) 27035-41. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB We analyzed the interaction of convulxin (Cvx), a 72-kDa protein isolated from the venom of Crotalus durissus terrificus, with human platelets. Cvx is a potent platelet agonist that induces an increase in the intracellular Ca2+ concentration ([Ca2+]i), granule exocytosis and aggregation. 125I-Labeled Cvx binds specifically and rapidly to platelets at binding sites of high and moderate affinity. Platelets adhere to immobilized Cvx in a time-dependent but cation-independent manner. Platelet exocytosis and aggregation induced by Cvx were inhibited by an anti-integrin alpha2beta1 monoclonal **antibody** (6F1) and by the Fab fragments of a polyclonal anti-glycoprotein VI (**GPVI**) **antibody**. Both the adhesion of platelets to Cvx and the Cvx-induced increase in [Ca2+]i were inhibited by anti-**GPVI** Fab fragments but not by 6F1. Ligand blotting assay showed that 125I-Cvx binds to a 57-kDa platelet protein with an electrophoretic mobility identical to that of **GPVI**. In addition, we observed the following: (i) 125I-Cvx binds to **GPVI** immunoprecipitated by the anti-**GPVI** **antibody** from a platelet lysate, and (ii) Cvx inhibits the binding of anti-**GPVI** IgG to **GPVI**. Taken together, these results demonstrate that

GPVI behaves as a Cvx receptor and that the alpha2beta1 integrin appears to be involved in the later stages of Cvx-induced platelet activation, i.e. exocytosis and aggregation.

L21 ANSWER 31 OF 44 MEDLINE DUPLICATE 22
97298057 Document Number: 97298057. PubMed ID: 9153205. Platelet activation and signal transduction by convulxin, a C-type lectin from Crotalus durissus terrificus (tropical rattlesnake) venom via the p62/**GPVI** collagen receptor. Polgar J; Clemetson J M; Kehrel B E; Wiedemann M; Magnenat E M; Wells T N; Clemetson K J. (Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-3012 Berne, Switzerland.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 May 23) 272 (21) 13576-83. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Convulxin, a powerful platelet activator, was isolated from Crotalus durissus terrificus venom, and 20 amino acid N-terminal sequences of both subunits were determined. These indicated that convulxin belongs to the heterodimeric C-type lectin family. Neither **antibodies** against GPIb nor echicetin had any effect on convulxin-induced platelet aggregation showing that, in contrast to other venom C-type lectins acting on platelets, GPIb is not involved in convulxin-induced platelet activation. In addition, partially reduced/denatured convulxin only affects collagen-induced platelet aggregation. The mechanism of convulxin-induced platelet activation was examined by platelet aggregation, detection of time-dependent tyrosine phosphorylation of platelet proteins, and binding studies with 125I-convulxin. Convulxin induces signal transduction in part like collagen, involving the time-dependent tyrosine phosphorylation of Fc receptor gamma chain, phospholipase Cgamma2, p72(SYK), c-Cbl, and p36-38. However, unlike collagen, pp125(FAK) and some other bands are not tyrosine-phosphorylated. Convulxin binds to a glycosylated 62-kDa membrane component in platelet lysate and to p62/**GPVI** immunoprecipitated by human anti-p62/**GPVI antibodies**. Convulxin subunits inhibit both aggregation and tyrosine phosphorylation in response to collagen. Piceatannol, a tyrosine kinase inhibitor with some specificity for p72(SYK), showed differential effects on collagen and convulxin-stimulated signaling. These results suggest that convulxin uses the p62/**GPVI** but not the alpha2beta1 part of the collagen signaling pathways to activate platelets. Occupation and clustering of p62/**GPVI** may activate Src family kinases phosphorylating Fc receptor gamma chain and, by a mechanism previously described in T- and B-cells, activate p72(SYK) that is critical for downstream activation of platelets.

L21 ANSWER 32 OF 44 MEDLINE DUPLICATE 23
96081829 Document Number: 96081829. PubMed ID: 7499287. Cyclic AMP-insensitive activation of c-Src and Syk protein-tyrosine kinases through platelet membrane glycoprotein VI. Ichinohe T; Takayama H; Ezumi Y; Yanagi S; Yamamura H; Okuma M. (Department of Internal Medicine, Faculty of Medicine, Kyoto University, Japan.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Nov 24) 270 (47) 28029-36. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Platelet glycoprotein (GP) VI is a so-far uncharacterized 62-kDa membrane protein, whose deficiency results in selective impairment in collagen-induced platelet aggregation. Our group previously reported a human polyclonal **antibody** (anti-p62 IgG) that induces activation of normal, but not of **GPVI**-deficient, platelets in an Fc-independent manner. The F(ab')2 fragments of this **antibody** (F(ab')2-anti-p62) stimulated tyrosine phosphorylation of numerous proteins, which was not prevented even in the presence of cAMP-increasing agents such as prostacyclin. Pretreatment of platelets with the protein-tyrosine kinase (PTK) inhibitor tyrphostin A47 completely abolished F(ab')2-anti-p62-induced platelet aggregation in parallel with dose-dependent inhibition of protein-tyrosine phosphorylation, indicating an essential requirement of PTK activity for generating **GPVI** -mediated signaling. We found that two cytosolic PTKs, c-Src and Syk,

became rapidly activated in response to F(ab')2-anti-p62 in a way insensitive to elevation of cAMP. In contrast, in the presence of prostacyclin, F(ab')2-anti-p62 did not stimulate tyrosine phosphorylation of the focal adhesion kinase. cAMP-insensitive activation of c-Src and Syk was also observed in collagen but not thrombin-stimulated platelets. Moreover, either F(ab')2-anti-p62 or collagen stimulated cAMP-insensitive tyrosine phosphorylation of phospholipase C-gamma 2. These results indicate that the receptor-mediated activation of several PTKs in platelets is regulated through a cAMP-sensitive or -insensitive mechanism depending on the nature of each stimulus, and also suggest that **GPVI** engagement is coupled to cAMP-insensitive activation of c-Src and Syk accompanied by tyrosine phosphorylation of numerous substrates including phospholipase C-gamma 2 in a manner similar to collagen stimulation.

L21 ANSWER 33 OF 44 CAPLUS COPYRIGHT 2002 ACS
1997:47369 Document No. 126:102203 Effect of unclustered platelet mAb on collagen-induced platelet aggregation and adherence to immobilized type I collagen fibrils. Tandon, Narendra N.; Matsuno, Kazuhiko; Jamieson, G. A. (UK). Leucocyte Typing V: White Cell Differ. Antigens, Proc. Int. Workshop Conf., 5th, Meeting Date 1993, Volume 2, 1218-1219. Editor(s): Schlossman, Stuart F. Oxford University Press: Oxford, UK. (English) 1995. CODEN: 63WDAC.
AB Platelet-collagen interaction is crit. for the initiation of hemostasis and is a complex process requiring multiple receptors. Although 14 putative collagen receptors have been described, only 3 (CD36, VLA-2, and **gpVI**) have been studied in detail. In order to investigate further the role of other putative receptors in platelet-collagen interaction, the authors examd. a panel of monoclonal **antibodies** for their effect on divalent cation-dependent and -independent platelet adhesion to immobilized collagen and on collagen-induced platelet aggregation.

L21 ANSWER 34 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
95:685934 The Genuine Article (R) Number: RP385. PLATELET MEMBRANE GLYCOPROTEIN-VI (**GPVI**) IS NECESSARY FOR COLLAGEN-INDUCED AGGREGATION AND ADHESION AND ANTI-GP-VI **ANTIBODY** INDUCES PLATELET-AGGREGATION - AN EVIDENCE OBTAINED FROM A PATIENT WITH SYSTEMIC LUPUS-ERYTHEMATOSUS. TAKAHASHI H (Reprint); HANANO M; MOROI M; SHIBATA A. KURUME UNIV, INST LIFE SCI, DEPT PROT CHEM, KURUME, FUKUOKA 830, JAPAN; NIIGATA UNIV, HOSP MED, DEPT BLOOD TRANSFUS, NIIGATA 95021, JAPAN; NIIGATA UNIV, HOSP MED, DEPT INTERNAL MED 1, NIIGATA 95021, JAPAN. THROMBOSIS AND HAEMOSTASIS (JUN 1995) Vol. 73, No. 6, pp. 1197. ISSN: 0340-6245. Pub. country: JAPAN. Language: ENGLISH.

L21 ANSWER 35 OF 44 MEDLINE DUPLICATE 24
95397362 Document Number: 95397362. PubMed ID: 7667837. Platelet unresponsiveness to collagen: involvement of glycoprotein Ia-IIa (alpha 2 beta 1 integrin) deficiency associated with a myeloproliferative disorder. Handa M; Watanabe K; Kawai Y; Kamata T; Koyama T; Nagai H; Ikeda Y. (Department of Blood Center, Keio University, Tokyo, Japan.) THROMBOSIS AND HAEMOSTASIS, (1995 Mar) 73 (3) 521-8. Journal code: VQ7; 7608063. ISSN: 0340-6245. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB We studied a 66-year-old man with a myeloproliferative disorder who presented with a prolonged bleeding time and marked thrombocytosis (platelet count, 3,890 x 10(9)/l). There was no past history of a bleeding disorder. The patient had normal coagulation data. His platelets completely lacked collagen-induced platelet aggregation and adhesion, but showed normal responses to other agonists. All family members tested showed normal platelet aggregation with collagen. Analysis of 125I surface-labeled platelets by two-dimensional SDS gel electrophoresis disclosed absence of the spot corresponding to platelet membrane GPIa (alpha 2) but no other significant deficiencies of major platelet glycoproteins i.e., GPIb, IIb-IIIa, and IV. Immunoisolation studies of the

patient's platelets indicated that neither anti-GPIa nor anti-GPIIa (beta 1) monoclonal **antibody** (mAb) isolated any surface membrane proteins corresponding to GPIa. **GPVI**, a putative collagen receptor, was immunoisolated from the platelets. Indirect immunofluorescence study using flow cytometry confirmed that the patient's platelets were totally deficient in surface expression of the GPIa-IIa complex (alpha 2 beta 1 integrin). In contrast, phytohemoagglutinin-activated T-lymphocytes from the patient expressed normal concentrations of this complex. The data suggest that our patient had an acquired deficiency of the platelet GPIa-IIa complex, due to a myeloproliferative disorder, which might account for the absence of responsiveness of his platelet to collagen.

L21 ANSWER 36 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
95:80020 The Genuine Article (R) Number: QB057. PLATELETS WITH 10-PERCENT OF THE NORMAL AMOUNT OF GLYCOPROTEIN-VI HAVE AN IMPAIRED RESPONSE TO COLLAGEN THAT RESULTS IN A MILD BLEEDING TENDENCY. ARAI M (Reprint); YAMAMOTO N; MOROI M; AKAMATSU N; FUKUTAKE K; TANOUE K. TOKYO MED COLL, DEPT CLIN PATHOL, SHINJUKU KU, 6-7-1 NISHISHINJUKU, TOKYO 160, JAPAN (Reprint); TOKYO METROPOLITAN INST MED SCI, DEPT CARDIOVASC RES, TOKYO 113, JAPAN; KURUME UNIV, INST LIFE SCI, DEPT PROT BIOCHEM, KURUME, FUKUOKA, JAPAN. BRITISH JOURNAL OF HAEMATOLOGY (JAN 1995) Vol. 89, No. 1, pp. 124-130. ISSN: 0007-1048. Pub. country: JAPAN. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Platelet glycoprotein VI (**GPVI**), a 62 kD membrane protein, has been identified as one of the platelet receptors for collagen, since **GPVI**-deficient platelets exhibit abnormal responses to collagen and an abnormal bleeding tendency. We report a female patient with a mild bleeding history whose platelets expressed 10% **GPVI** of normal platelets. Shape change, aggregation and ATP release of the patient's platelets were completely absent in response to 1-5 mu g/ml collagen but present normally in response to ADP and Ca2+ ionophore A23187. Adhesion of the patient's platelets to coated collagen was mildly affected (40-60% of normal platelets) in spite of only 10% expression of **GPVI**. Flow cytometrical studies revealed that the patient's platelets expressed normal amounts of the GPIa/IIa complex. These results suggest that platelet **GPVI** is less involved in adhesion to collagen than shape change and aggregation induced by collagen.

L21 ANSWER 37 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
96083913 EMBASE Document No.: 1996083913. Clinical and serological manifestations of genital human papillomavirus infection. Wikstrom A.. Department of Dermatovenereology, Karolinska Hospital,Stockholm, Sweden. Acta Dermato-Venereologica, Supplement -/193 (I-85) 1995. ISSN: 0365-8341. CODEN: AVSUAR. Pub. Country: Norway. Language: English. Summary Language: English.
AB Efficacy of chemical and/or surgical treatment for penile and anal condylomata acuminata was investigated in two retrospective studies of hetero- and homosexual men. Variation in clinical features and symptomatology as well as the reliability of diagnostic criteria by different methods for acetowhite penile lesions was also studied. Furthermore, the **antibody** response in the course of penile wart disease as well as in asymptomatic genitoanal papillomavirus infection (**GPVI**) was analysed. In the first retrospective study, as much as 23% of patients still had condylomas after one year of chemical and/or surgical treatment. On the other hand, 38% were cured after a single treatment session. In the group mainly with anal warts, concurrent penile warts were significantly more common among heterosexual men compared to homosexual men ($p < 0.001$), while intra-anal wart growth was more common among the homosexual males ($p < 0.001$). When comparing diagnostic methods for subclinical penile HPV infection, conventional histopathology appeared to be the most valuable diagnostic aid to penoscopy, while the additional use of Southern blot, in situ hybridization and PCR assays for HPV DNA detection did not increase the predictive value of **GPVI**. We also describe a new distinct clinical entity, HPV-associated balanoposthitis,

comprising a wide range of often long-lasting symptoms, such as itching, burning and dyspareunia. A significant increase in the IgG **antibody** response against defined epitopes in the L1 and L2 capsid proteins of HPV 6, was found among men with previous condylomata. By following a cohort of STD clinic patients with multiple brush samples from the genitoanal region as well as serum samples taken at several consecutive clinical visits, we identified 16 patients who had seroconverted to HPV seropositivity during follow-up. **Antibody** responses to several HPV-derived peptide and protein antigens were induced at the same time. Seroconversions were usually seen concomitantly with HPV acquisition or at the vist after HPV DNA was first detected. The HPV **antibody** response was frequently transient and declined or disappeared after clearance of infection. The **antibody** responses were induced by several different HPV types, indicating limited type-specificity. The most type-restricted response was against HPV 16 capsids, where seroconversions to continuous seropositivity were induced by infection with HPV 16.

L21 ANSWER 38 OF 44 MEDLINE DUPLICATE 25
96282020 Document Number: 96282020. PubMed ID: 8721519. Clinical and serological manifestations of genital human papillomavirus infection. Wikstrom A. (Department of Dermatovenereology, Karolinska Hospital, Stockholm, Sweden.) ACTA DERMATO-VENEREOLOGICA. SUPPLEMENTUM, (1995) 193 1-85. Journal code: 0MS; 0370311. ISSN: 0365-8341. Pub. country: Norway. Language: English.

AB Efficacy of chemical and/or surgical treatment for penile and anal condylomata acuminata was investigated in two retrospective studies of hetero- and homosexual men. Variation in clinical features and symptomatology as well as the reliability of diagnostic criteria by different methods for acetowhite penile lesions was also studied. Furthermore, the **antibody** response in the course of penile wart disease as well as in asymptomatic genitoanal papillomavirus infection (**GPVI**) was analysed. In the first retrospective study, as much as 23% of patients still had condylomas after one year of chemical and/or surgical treatment. On the other hand, 38% were cured after a single treatment session. In the group mainly with anal warts, concurrent penile warts were significantly more common among heterosexual men compared to homosexual men ($p < 0.001$), while intra-anal wart growth was more common among the homosexual males ($p < 0.001$). When comparing diagnostic methods for subclinical penile HPV infection, conventional histopathology appeared to be the most valuable diagnostic aid to penoscopy, while the additional use of Southern blot, in situ hybridisation and PCR assays for HPV DNA detection did not increase the predictive value of **GPVI**. We also describe a new distinct clinical entity, HPV-associated balanoposthitis, comprising a wide range of often long-lasting symptoms, such as itching, burning and dyspareunia. A significant increase in the IgG **antibody** response against defined epitopes in the L1 and L2 capsid proteins of HPV 6, was found among men with previous condylomata. By following a cohort of STD clinic patients with multiple brush samples from the genitoanal region as well as serum samples taken at several consecutive clinical visits, we identified 16 patients who had seroconverted to HPV seropositivity during follow-up. **Antibody** responses to several HPV-derived peptide and protein antigens were induced at the same time. Seroconversions were usually seen concomitantly with HPV acquisition or at the visit after HPV DNA was first detected. The HPV **antibody** response was frequently transient and declined or disappeared after clearance of infection. The **antibody** responses were induced by several different HPV types, indicating limited type-specificity. The most type-restricted response was against HPV 16 capsids, where seroconversions to continuous seropositivity were induced by infection with HPV 16.

L21 ANSWER 39 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
96:242120 The Genuine Article (R) Number: UA890. CLINICAL AND SEROLOGICAL MANIFESTATIONS OF GENITAL HUMAN PAPILLOMAVIRUS INFECTION. WIKSTROM A

(Reprint). KAROLINSKA HOSP, DEPT DERMATOVEREREOL, S-10401 STOCKHOLM, SWEDEN (Reprint); KAROLINSKA INST, MICROBIOL & TUMORBIOL CTR, STOCKHOLM, SWEDEN. ACTA DERMATO-VENEREOLOGICA (1995) Supp. 193, pp. 1. ISSN: 0001-5555. Pub. country: SWEDEN. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Efficacy of chemical and/or surgical treatment for penile and anal condylomata acuminata was investigated in two retrospective studies of hetero- and homosexual men. Variation in clinical features and symptomatology as well as the reliability of diagnostic criteria by different methods for acetowhite penile lesions was also studied. Furthermore, the **antibody** response in the course of penile wart disease as well as in asymptomatic genitoanal papillomavirus infection (**GPVI**) was analysed.

In the first retrospective study, as much as 23% of patients still had condylomas after one year of chemical and/or surgical treatment. On the other hand, 38% were cured after a single treatment session. In the group mainly with anal warts, concurrent penile warts were significantly more common among heterosexual men compared to homosexual men ($p<0.001$), while intra-anal wart growth was more common among the homosexual males ($p<0.001$). When comparing diagnostic methods for subclinical penile HPV infection, conventional histopathology appeared to be the most valuable diagnostic aid to penoscopy, while the additional use of Southern blot, in situ hybridisation and PCR assays for HPV DNA detection did not increase the predictive value of **GPVI**. We also describe a new distinct clinical entity, HPV-associated balanoposthitis, comprising a wide range of often long-lasting symptoms, such as itching, burning and dyspareunia.

A significant increase in the IgG **antibody** response against defined epitopes in the L1 and L2 capsid proteins of HPV 6, was found among men with previous condylomata. By following a cohort of STD clinic patients with multiple brush samples from the genitoanal region as well as serum samples taken at several consecutive clinical visits, we identified 16 patients who had seroconverted to HPV seropositivity during follow-up. **Antibody** responses to several HPV-derived peptide and protein antigens were induced at the same time. Seroconversions were usually seen concomitantly with HPV acquisition or at the visit after HPV DNA was first detected. The HPV **antibody** response was frequently transient and declined or disappeared after clearance of infection. The **antibody** responses were induced by several different HPV types, indicating limited type-specificity. The most type-restricted response was against HPV 16 capsids, where seroconversions to continuous seropositivity were induced by infection with HPV 16.

L21 ANSWER 40 OF 44 CAPLUS COPYRIGHT 2002 ACS
1992:253867 Document No. 116:253867 Specific lysis of targets expressing varicella-zoster virus gpI or gpIV by CD4+ human T-cell clones. Huang, Z.; Vafai, A.; Lee, J.; Mahalingam, R.; Hayward, A. R. (Dep. Pediatr., Univ. Colorado, Denver, CO, 80262, USA). J. Virol., 66(5), 2664-9 (English) 1992. CODEN: JOVIAM. ISSN: 0022-538X.

AB Varicella-zoster virus (VZV)-specific CD4-pos. T cells are known to lyse targets expressing VZV antigen, but little is known of the glycoprotein specificity or phenotype of these cells. To test the ability of T cells to distinguish between glycoproteins gpI and gpIV (which share an **antibody**-defined epitope), clones were prepd. from blood from healthy individuals by limiting diln. Among 68 T-cell clones which were VZV specific in tests of proliferation, 30 lysed autologous Epstein-Barr virus-transformed lymphoblasts which had been superinfected with a recombinant vaccinia virus which included the whole VZV gpI sequence. These clones were characterized as major histocompatibility complex class II restricted by inhibition of their cytotoxicity with HLA-DR and CD4 monoclonal **antibodies**. Twenty-one clones lysed targets expressing gpIV. Fifteen of these clones lysed targets expressing gpI and gpIV. Four clones with gpI-gpIV specificity were examd. in detail, and their dual specificity was confirmed by cold target inhibition. These 4 clones failed to kill target cells infected with a mutant gpIV recombinant vaccinia virus from which amino acid residues 212-354 had been deleted.

This region includes one of the two gpIV decapeptides which have 50% homol. with amino acids 111-121 of gpI. Thus, T-cell-receptor-assocd. structures are required for specific lysis of VZV targets and (i) gpI-specific CD4 cytotoxic T cells outnumber gpIV-specific T cells in blood and (ii) 50% of gpI-specific T-cell clones also lyse gpIV-expressing targets.

L21 ANSWER 41 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1992:451168 Document No.: BA94:92568. PLATELET-AGGLUTINATING PROTEIN P37 FROM A THROMBOTIC THROMBOCYTOPENIC PURPURA PLASMA FORMS COMPLEXES WITH PLATELET MEMBRANE GLYCOPROTEIN IV CD36. SIDDIQUI F A; LIAN E C-Y. HEMOPHILIA TROMBOSIS CENT., W.J. HARRINGTON CENT. BLOOD DISEASE, UNIV. MIAMI SCH. MED., MIAMI, FLA.. BIOCHEM INT, (1992) 27 (3), 485-496. CODEN: BIINDF. ISSN: 0158-5231. Language: English.

AB We have previously reported the purification of a 37 kDa platelet agglutinating protein (PAPp37) from the plasma of a patient with Thrombotic Thrombocytopenic purpura (TTP), and have shown recently that p37 causes platelet agglutination through its binding to membrane glycoprotein IV (GPIV). To gain further insight into the mechanism of p37 binding to **GPVI**, we have studied the interaction between p37 and GPIV. We not demonstrate specific complex formation of p37 with GPIV. In Western immunoblotting p37 binds to purified GPIV and the complex formed between the two proteins was detected by polyclonal **antibody** to p37 and peroxidase conjugated second **antibody**. No binding of p37 was noticed with the purified GPIIIa. A solid phase binding assay was developed to study the complex formation. Microtiter wells were coated with GPIV and the control proteins; 125I-p37 was added, allowed to bind and bound radioactivity was measured. Several lines of evidence indicate that the binding of p37 to GPIV was specific. a) GPIV immobilized on Immulon-2 wells bound 10-30 fold more p37 than immobilized fibrinogen, GPIIIa, and BSA. b) Polyclonal **antibodies** against p37 and GPIV inhibited the binding by 39-68% as compared with control IgG. c) GPIIIa **antibody** did not inhibit the binding. Molecular sieve chromatography of a mixture of 125I-p37 and GPIV also revealed the fluid phase complex formation ranging in molecular weight from 132,000 to over 350,000 daltons. These results show the specific interaction between p37 and GPIV and suggest that GPIV functions as a p37 receptor during platelet agglutination.

L21 ANSWER 42 OF 44 MEDLINE DUPLICATE 26
93003333 Document Number: 93003333. PubMed ID: 1390897. Platelet adhesion to collagen-coated wells: analysis of this complex process and a comparison with the adhesion to matrigel-coated wells. Moroi M; Okuma M; Jung S M. (Department of Protein Biochemistry, Kurume University, Japan.) BIOCHIMICA ET BIOPHYSICA ACTA, (1992 Oct 6) 1137 (1) 1-9. Journal code: A0W; 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB The mechanisms of platelet adhesion to collagen type III-coated wells and Matrigel-coated wells were analyzed. The adhesion of 51Cr-labeled platelets to collagen-coated wells showed a biphasic pattern. The early stage of adhesion was inhibited by **antibodies** against platelet glycoprotein(GP)s Ia/IIa and VI. The later stage of platelet adhesion was inhibited by an **antibody** against the GPIIb/IIIa complex and a concomitant release of 14C-labeled serotonin was observed. The percentage of adhered platelets was increased when a higher platelet concentration was added in the reaction medium. These results indicated that the adhesion assay of platelets to collagen-coated wells was composed of two reactions: the first one is the platelet-collagen interaction that depends on GPIa/IIa and **GPVI** on the platelet surface; and the second reaction is the platelet-platelet interaction, platelet aggregation, which depends on GPIIb/IIIa. The adhesion of platelets to Matrigel-coated wells was indicated to involve platelet-Matrigel interactions that were partly dependent on the laminin in the Matrigel solution.

L21 ANSWER 43 OF 44 MEDLINE DUPLICATE 27

92144690 Document Number: 92144690. PubMed ID: 1782228. Genitoanal papillomavirus infection: diagnostic and therapeutic objectives in the light of current epidemiological observations. von Krogh G. INTERNATIONAL JOURNAL OF STD AND AIDS, (1991 Nov-Dec) 2 (6) 391-404. Ref: 154. Journal code: A16; 9007917. ISSN: 0956-4624. Pub. country: ENGLAND: United Kingdom. Language: English.

AB During the past decade a wide span of heterogeneity has been demonstrated for human papillomaviruses (HPVs), and some basic properties of the HPV genome have been revealed. The use of hybridization assays for HPV DNA detection in infected epithelia, and the recent introduction of synthetic HPV peptides for detection of type-specific circulating **antibodies** , have resulted in a major rethinking of HPV epidemiology. Recent data indicate that various HPVs may be transmitted perinatally during early infancy and that a long latency with periodic reactivation seems to be quite common. The present review attempts to assess recent epidemiological data with the concept of genitoanal papillomavirus infection (**GPVI**) as a predominantly sexually transmitted disease. Some diagnostic and therapeutic aspects are outlined with a pragmatic approach to the clinical relevance of **GPVI**.

L21 ANSWER 44 OF 44 MEDLINE DUPLICATE 28

90037566 Document Number: 90037566. PubMed ID: 2808700. A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. Moroi M; Jung S M; Okuma M; Shinmyozu K. (Department of Biochemistry II, Jichi Medical School, Tochigi, Japan.) JOURNAL OF CLINICAL INVESTIGATION, (1989 Nov) 84 (5) 1440-5. Journal code: HS7; 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Molecular level studies on platelets deficient in collagen-induced aggregation provide evidence for identifying possible platelet collagen receptors. We investigated platelets from a patient with mild bleeding time prolongation, but otherwise normal coagulation data. Her platelets lacked collagen-induced aggregation and adhesion, but retained normal aggregation and release by other agonists. Labeling her platelets with 125I or 3H and analysis by SDS-PAGE/autoradiography showed normal levels of glycoproteins Ia, Ib, IIa, IIb, IIIa, and IV. However, there were significantly decreased incorporations of both radioactivities into a 61-kD membrane glycoprotein (GP), which was identified as **GPVI** from its mobility on unreduced-reduced, two-dimensional SDS-PAGE. Sugiyama et al. (1987. Blood. 69: 1712) reported that the serum from an idiopathic thrombocytopenic purpura (ITP) patient contained an **antibody** against a 62-kD platelet protein. Our patient's platelets lacked the antigen for the ITP patient's **antibody**, demonstrating that the ITP serum contains a specific **antibody** against **GPVI**. The patient's parents' platelets contained approximately 50% the normal amount of **GPVI**, but still had normal collagen-induced aggregation and adhesion. The patient's platelets did not bind to types I and III collagen fibrils. Our results suggest that **GPVI** functions as a collagen receptor.

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